Abca7 Null Mice Retain Normal Macrophage Phosphatidylcholine and Cholesterol Efflux Activity despite Alterations in Adipose Mass and Serum Cholesterol Levels*

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Mutations in the A class of ATP-binding cassette transporters (ABCA) are causally implicated in three human diseases: Tangier disease (ABCA1), Stargardt’s macular degeneration (ABCA4), and neonatal respiratory failure (ABCA3). Both ABCA1 and ABCA4 have been shown to transport lipids across cellular membranes, and ABCA3 may play a similar role in transporting pulmonary surfactant. Although the functions of the other 10 ABCA class transporters identified in the human genome remain obscure, ABCA7-transfected cells have been shown to efflux lipids in response to stimulation by apolipoprotein A-I. In an effort to elucidate the physiologic role of ABCA7, we generated mice lacking this transporter (Abca7−/− mice). Homozygous null mice were produced from intercrosses of heterozygous null mice at the expected Mendelian frequency and developed normally without any obvious phenotypic abnormalities. Cholesterol and phospholipid efflux stimulated by apolipoprotein A-I from macrophages isolated from wild type and Abca7−/− mice did not differ, suggesting that these activities may not be central to the physiologic role of the transporter in vivo. Abca7−/− females, but not males, had significantly less visceral fat and lower total serum and high density lipoprotein cholesterol levels than wild type, gender-matched littermates. ABCA7 expression was detected in hippocampal and cortical neurons by in situ hybridization and in brain and white adipose tissue by Western blotting. Induction of adipocyte differentiation from 3T3 fibroblasts in culture led to a marked increase in ABCA7 expression. These studies suggest that ABCA7 plays a novel role in lipid and fat metabolism that Abca7−/− mice can be used to elucidate.

ATP-binding cassette (ABC) transporters form a large and ancient gene family, many members of which have been linked to genetic diseases in humans (1). The ABC transporter family is currently divided into seven classes (A–G) with the A and C classes containing the largest number of members (12 each). A and C class transporters are full transporters that contain two ATP-binding cassettes and two multiple-membrane-spanning domains encoded by a single cistron. The A class of transporters shares considerable sequence homology that we and others have shown leads to a topological configuration in which the two largest loops of the protein are positioned on the exoplasmic side of the plasma membrane (2–4). Studies of ABCA1 and ABCA4 have demonstrated that the transporters are involved in an ATP-dependent movement of phospholipids across cellular membranes (5, 6). ABCA1 also transports cholesterol, leading to net cholesterol export to apolipoprotein acceptors, such as apoA-I or apoE (7, 8). Defects in this pathway lead to intracellular cholesterol accumulation and cause Tangier disease in humans (9–11). Mutations in ABCA4 cause Stargardt’s macular degeneration, a disorder characterized by abnormal lipid accumulation in the retinal pigment epithelium (12–14). Recently, children suffering from neonatal respiratory failure and defective pulmonary surfactant secretion were found to have genetic mutations mapping to the ABCA3 gene locus (15). Thus, based on sequence homology and functional activity, it seems likely that all of the ABCA class transporters will play a role in lipid trafficking in one or more tissues.

Of the 12 A class members, ABCA7 and ABCA1 are among the most closely related, sharing 50% sequence identity. Recent studies in transfected cell lines have indicated that ABCA7 also shares the functional attributes of ABCA1 of phospholipid and cholesterol transport in response to stimulation with apoA-I, although the magnitude of the sterol transport activity is disrupted (16, 17). Like ABCA1, ABCA7 is widely expressed in vertebrate tissues with the highest levels reported in brain, lung, macrophages, and platelets (16, 18). Liver expression of ABCA7, however, does not mimic that seen with ABCA1. Because the loss of ABCA1 alone in mice or humans leads to a dramatic decline in circulating HDL levels and a near absence of apoA-I-stimulated cholesterol efflux, it is clear that the activity of ABCA7 cannot compensate for the loss of ABCA1. Thus, the significance of the lipid efflux measured in ABCA7-transfected cells is uncertain, and the physiologic role of the transporter is unknown.

To explore this function, we generated Abca7−/− null mice and performed an initial characterization of their phenotype. From intercrosses of heterozygous Abca7−/− mice, homozygous null animals were born at the expected one in four frequency. In controlled feeding studies, these mice fed normally and gained weight in a manner indistinguishable from their wild type littermates. However, female Abca7−/− mice had lower white adipose mass than their wild type littermates and lower

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total serum and HDL cholesterol levels. These serum lipid differences were much less profound than those seen in comparisons of wild type and Abca1−/− mice. We also found no disruption in macrophase efflux of phosphatidylecholine or cholesterol efflux to apoA-1, as might have been predicted from the published Abca7 cell transfection experiments. These results indicate that Abca7 does not recapitulate ABCA1 function but rather has distinct activities that will require further investigation of wild-type and null mice to elucidate.

**Materials and Methods**

*Generation of Abca7 Null Mice*—129/SvEv mouse genomic DNA was PCR-amplified using primers (5’-GGGCGGCAGCTGTGATTTGCAGCCCTAAAG-3’ and 5’-GGGGGCGGACACATAGCAACAGAACTCAGCTG-3’) to generate 5.2- and 4.6-kb fragments that encompassed exons 9–19 and 22–33 of Abca7. Using introduced restriction sites (Asci, NotI and BseI, respectively), the 5.2 and 4.6-kilobase pair products were cloned into the KO targeting vector (19). The resulting targeting vector was linearized and electroporated into J1 embryonic stem cells derived from 129/SvEv mice. After selecting against nonhomologous recombinants, 180 G418/gancyclovir-resistant clones were selected and expanded. Genomic DNA from these clones was restricted with SpeI/DraI, separated on 0.7% agarose gels, and transferred to hybridization membranes (Gene Screen Plus; PerkinElmer Life Sciences). The membranes were probed with a radiolabeled cDNA fragment encompassing exons 9–15 of the Abca7 cDNA (nucleotides 217–815). This probe detects an 11.2-kb fragment in the endogenous Abca7 allele and an 8.0-kb fragment in the targeted allele. Correctly targeted embryonic stem cells were microinjected into C57BL/6 blastocysts to generate chimeric mice that were back-crossed into the C57BL/6 background to obtain heterozygous Abca7+/− null mice. These animals were then intercrossed to produce Abca7/− mice, as well as wild type littermates.

*Animal Procedures and Lipid Analysis*—C57BL/6 and 129/Sv mice were purchased from Charles River Laboratories (Wilmington, MA). All of the animal procedures were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care and were conducted in accordance with the United States Department of Agriculture Animal Welfare Act and the Public Health Service Policy for the Humane Care and Use of Laboratory Animals. The mice were housed under pathogen-free conditions on a standard 12-h light/dark cycle and were given free access to a standard chow diet and water. For feeding and weight gain measurements, after weaning mice at 4 weeks of age, the mice were housed singly and monitored for food intake and weight gain every 3 days. At 10 weeks of age, the mice were fasted overnight and anesthetized, and blood samples were drawn by the retro-orbital route. Serum was collected and cultured for an additional 6 days to obtain mature macrophages.

**Generation of Abca7 Null Mice**—Bone marrow-derived macrophages were prepared from wild type and Abca7+/− mice as described previously (20). Femurs were flushed with cold Dulbecco’s modified Eagle’s medium, and bone marrow was collected by centrifugation at 2200 rpm. Red blood cells were lysed with 0.17 M NH4Cl, and bone marrow was resuspended in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 15% L929-conditioned medium. After 24 h, the nonadherent cells were collected and cultured for an additional 6 days to obtain mature macrophages.

**Abca7 cDNA and Antibody**—The mouse Abca7 cDNA was amplified by reverse transcription-PCR from a macrophase cDNA library, and its sequence was verified on both strands by dideoxy sequencing. The Abca7 and Abca1 cDNAs were subcloned into the pcDNA3.1 vector downstream of the cytomegalovirus promoter. An anti-Abca7 antisense was raised against the C-terminal 128 amino acids of mouse Abca7 cDNA (21). The specificity of the sera obtained was confirmed by immunoblotting of lysates from 293 cells transfected with either Abca7 or Abca1 cDNAs using either Abca7 antisense (1:500 dil) or preimmune control serum (1:500).

**Western Immunoblotting**—Membrane protein, as determined by BCA assays, was separated by SDS-PAGE and its sequence was verified on both strands by dideoxy sequencing. The ABCA7 cDNA and Antibody—The mouse Abca7 cDNA was amplified by reverse transcription-PCR from a macrophase cDNA library, and its sequence was verified on both strands by dideoxy sequencing. The Abca7 and Abca1 cDNAs were subcloned into the pcDNA3.1 vector downstream of the cytomegalovirus promoter. An anti-Abca7 antisense was raised against the C-terminal 128 amino acids of mouse Abca7 cDNA (21). The specificity of the sera obtained was confirmed by immunoblotting of lysates from 293 cells transfected with either Abca7 or Abca1 cDNAs using either Abca7 antisense (1:500 dil) or preimmune control serum (1:500).

**Western Immunoblotting**—Membrane protein, as determined by BCA assays, was separated by SDS-PAGE and transferred to a nitrocellulose membrane using a Trans-Blot System (Bio-Rad). The membranes were blocked overnight at 4 °C in 1× PBS, 5% nonfat dry milk, and 1% bovine serum albumin, and were probed with the anti-Abca7 or anti-Abca1 antisense diluted 1:500 at room temperature for 2 h. The membranes were washed three times with 1× PBS containing 0.1% Tween 20 and then incubated with horseradish peroxidase-conjugated goat anti-rabbit antibody (Sigma) for 2 h. Western immunoblotting signals were detected using the enhanced chemiluminescence Super Signal (Pierce) and x-ray films.

**In Situ Hybridization**—In situ hybridization was performed using the Radiopharmaceutical System (New England Nuclear, Boston, MA) according to the manufacturer’s protocol. Briefly, the mouse brain was harvested following perfusion with 4% paraformaldehyde, and paraffin-embedded tissue slides were prepared. To generate the probes, a fragment of Abca7 cDNA (214–815 bp) was cloned into the pBluescript vector (Stratagene). Sense and antisense riboprobes were generated using digoxigenin-labeled UTP. Hybridization was performed at 65 °C for 6 h, and the slides were washed in 0.1× SSC at 75 °C for 6 min. The hybridized probe was detected with biotinylated anti-digoxigenin antibody, streptavidin–alkaline phosphatase conjugate, and the substrate nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate. The slides were counterstained with nuclear Fast Red.

**Cholesterol and Phospholipid Efficacy Assays**—Studies of cholesterol and phospholipid efflux were performed in bone marrow-derived macrophages or 293 and 293-EBNA T cells transfected with Abca7 or Abca1 cDNAs as we have described previously (22). In brief, cells in 24-well plates were loaded with 0.5 μCi/ml [3H]cholesterol or 1 μCi/ml [3H]methyl-choline for 24 h, washed twice with warm PBS, incubated for 2 h in Dulbecco’s modified Eagle’s medium (1 mg/ml fatty acid free bovine serum albumin), and then washed twice with warm PBS. The cells were then incubated in the presence of 10 μg/ml LDL, 0.1 μg/ml HDL, or medium alone for 20 h. Medium was collected and cleared of cellular debris by 800 × g spin for 10 min. For cholesterol efflux, the cell layers were dissolved in 0.1× NaOH, and the amount of radioactive in the media and cell lysates were measured by scintillation counting. For phosphatidylethanolamine efflux, the media and cell lysates were subjected to hexane/isopropanol (3:2 v/v) extraction, and the radioactivity in the organic phase was determined by scintillation counting as described...
previously and expressed as a percentage of efflux (media cpm/(media + cell cpm) × 100) (23). To load cells with excess cholesterol and activate the LXR/RXR transcriptional network, cholesterol (10 μg/ml), 22-R-hydroxycholesterol (10 μM), and 9-cis-retinoic acid (10 μM) were added to the cells at the beginning of the labeling step.

For comparison of ABCA7 and ABCA1 phospholipid efflux, the data were normalized to cell surface expression using a 9-amino acid FLAG tag (DYKDDDDK) introduced after amino acid position 155 of the mouse ABCA7 cDNA or 207 of ABCA1, as we have described previously (22). Comparison of wild type and FLAG-ABCA7 transporters demonstrated that this tag had no measurable impact on protein expression and phospholipid efflux.

RESULTS

Generation of ABCA7−/− Mice—The targeting strategy to produce mice lacking ABCA7 is shown in Fig. 1A. Exons 20 and 21 were targeted for deletion because they encode the signature and Walker B motifs of the first ATP-binding domain, elements that are known to be critical for ABCA1 function. The splicing of exons 19–22 would be predicted to cause a frame shift mutation, leading to a stop codon encoded by nucleotides 9–11 of exon 22. The targeting vector was linearized and electroporated into 129/SvEv embryonic stem cells. After positive and negative antibiotic selections, 180 clones were selected and screened for homologous recombination at the Abca7 locus. Nine clones were confirmed by PCR and Southern analysis to be correctly targeted, giving a targeting efficiency of 5%. Embryonic stem cells from two clones were expanded and injected into C57BL/6 blastocysts, resulting in chimeric mice that transmitted the targeted allele (Fig. 1, B and C). Mating of heterozygous offspring of the chimeric mice produced Mendelian frequencies of the three expected genotypes (wild type:heterozygous:null, 60:102:55).

ABCA7 Expression and Regulation—To confirm that homologous recombination had disrupted ABCA7 protein expression,
immunoblots of tissue lysates were performed with an ABCA7 antiserum that we generated. The specificity of this ABCA7 antiserum was first validated, given the sequence similarity of the ABCA1 and ABCA7 transporters. This antiserum bound ABCA7 but did not cross-react with ABCA1 (Fig. 2A). Similarly, the antiserum we previously raised to ABCA1 did not

**Fig. 3.** Abca7−/− mice feed and gain weight normally. Female wild type (n = 4) and null littermates (n = 4) were individually housed, and food intake on a standard chow diet was assessed for a 6-week period (A). Weight gain for these same mice was also measured (B). The error bars represent the standard error of the mean.

**TABLE I**

Weights of wild type and Abca7−/− mice and major organs at 10 weeks of age

|            | Female |                  | Male |                  |
|------------|--------|------------------|------|------------------|
|            | Wild type (n = 13) | Null (n = 13) | t test | Wild type (n = 11) | Null (n = 11) | t test |
| Total body | 20.65 ± 1.93 | 19.91 ± 2.01 | 0.33 | 27.11 ± 2.98 | 26.66 ± 3.37 | 0.75 |
| Brain      | 0.47 ± 0.06 | 0.43 ± 0.07 | 0.14 | 0.42 ± 0.04 | 0.40 ± 0.03 | 0.61 |
| Lung       | 0.18 ± 0.05 | 0.16 ± 0.04 | 0.29 | 0.17 ± 0.06 | 0.15 ± 0.04 | 0.37 |
| White adipose | 0.47 ± 0.27 | 0.28 ± 0.18 | 0.04 | 0.52 ± 0.23 | 0.54 ± 0.04 | 0.86 |
| Heart      | 0.11 ± 0.03 | 0.11 ± 0.01 | 0.48 | 0.15 ± 0.02 | 0.15 ± 0.03 | 0.67 |
| Liver      | 0.84 ± 0.14 | 0.82 ± 0.08 | 0.59 | 1.04 ± 0.39 | 1.07 ± 0.15 | 0.83 |
| Kidney     | 0.28 ± 0.03 | 0.29 ± 0.03 | 0.39 | 0.40 ± 0.04 | 0.36 ± 0.15 | 0.03 |
| Spleen     | 0.07 ± 0.02 | 0.06 ± 0.02 | 0.47 | 0.08 ± 0.03 | 0.08 ± 0.15 | 0.54 |

**Fig. 4.** Female Abca7−/− mice have lower total and HDL cholesterol levels. Serum total cholesterol (A) and triglycerides (B) were determined on littermate wild type (WT) and null animals of both genders (n = 6, each group). Total cholesterol levels were significantly lower in the female null animals (t test, p = 0.017, as indicated by the asterisk). Pooled plasma from these mice was subjected to fast protein liquid chromatography fractionation, and the cholesterol content in the fractions was measured (C and D). ApoA-I (E) and apoE (F) content was assessed by immunoblotting of the indicated fractions taken from female mouse sera.
recognize ABCA7 expressed by transfected 293 cells (Fig. 2A). By Western blotting, we confirmed that the deletion of exons 20 and 21 had indeed abrogated ABCA7 protein expression in mice homozygous for the targeted allele (Fig. 2B). ABCA1 levels, as detected by immunoblotting, did not appear to be altered in the Abca7−/− null mouse, suggesting that there is no compensation by ABCA1 for the loss of the activity of ABCA7 (Fig. 2B).

As we observed the highest level of ABCA7 tissue expression in the brain, we further characterized its localization in that tissue. Using in situ hybridization analysis, strong neuronal staining was evident throughout the brain using an antisense probe, whereas the sense probe from the same construct yielded no signal. Staining is particularly evident in the densely packed neurons of the hippocampus (Fig. 2C).

A second site of robust ABCA7 expression was the white adipose tissue. We assessed whether ABCA7 expression was up-regulated during adipocyte differentiation using a 3T3L1 adipocyte differentiation model. Upon differentiation of 3T3L1 preadipocytes to mature adipocyte-like cells, ABCA7 expression was greatly increased, suggesting that this protein may play a role in lipid transport in these cells (Fig. 2D). Subcellular fractionation showed that the ABCA7 transporter colocalized in part with perilipin in the lipid droplet and microsomal fractions, a distribution clearly distinct from that of ABCA1 (Fig. 2D).

**Feeding Behavior and Weight Gain Analysis of Abca7 Null Mice**—Having established that we had generated mice lacking ABCA7 expression and produced evidence for its potential involvement in fat metabolism, we next examined the impact of the loss of the transporter on parameters of growth and metabolism. In these studies, littersize, gender-matched wild type and null mice were compared. Abca7−/− mice fed and gained weight similarly to their wild type counterpart (Fig. 3). At 10 weeks of age, internal organs were found to be of similar weights in both groups (Table I), with the exception of female white adipose tissue and male kidneys, both of which were found to be smaller in Abca7−/− mice. Although the differences in kidney size were quite modest (≤ 10%), the adipose mass of the Abca7−/− female mice was approximately half that of the wild type females. Despite the reduction in size of both of these tissues, histologic examination failed to reveal any overt pathology (data not shown).

**Quantification of Serum and Tissue Lipids in Abca7 Null Mice**—We next tested whether the loss of ABCA7 expression significantly affected serum cholesterol, triglycerides, and free fatty acid levels. Total triglycerides were similar in wild type and Abca7−/− mice of both genders; however, total cholesterol was significantly lower in female Abca7−/− mice (Fig. 4, A and B). Serum was fractionated by fast protein liquid chromatography, and the cholesterol was measured in each fraction to determine the source of the cholesterol difference in the Abca7−/− females. The reduction in total cholesterol levels could be accounted for by a drop in the cholesterol carried in the HDL peak (Fig. 4C). Male wild type and Abca7−/− mice, whose total cholesterol levels were similar, had similar cholesterol levels across the lipoprotein fractions, as expected (Fig. 4D). Immuno blotting of the lipoprotein fractions from females showed that despite a drop in cholesterol content, apoa-I and ApoE protein levels were similar in the fractions from wild type and Abca7−/− mice (Fig. 4, E and F), indicating that no major redistribution of these apoproteins had occurred in the serum lipoproteins. Having found significant ABCA7 expression in white adipose tissue, we also measured the serum levels of free fatty acids by gas chromatography-coupled mass spectrometry to determine whether they were altered by the absence of the transporter. In both genders, the levels and distribution of serum-free fatty acids did not appear to be altered in Abca7−/− mice (Fig. 5).

**ABCA7−/− Macrophage Efflux of Lipid to ApoA-I and HDL Is Normal**—The decline in HDL cholesterol in females, coupled with data from previous studies on ABCA7 activity in transfected cells, suggested that apolipoprotein or HDL stimulated cholesterol efflux might be impaired in Abca7−/− macrophages. To test this hypothesis, we isolated bone marrow macrophages from wild type mice and verified that ABCA7 was expressed in these cells under our conditions of isolation and proliferation (Fig. 6A). Stimulation of these cells with cholesterol loading and LXR/RXR agonist treatment markedly up-regulated ABCA1, as expected, but had little or no impact on ABCA7 expression (Fig. 6A). ApoA-I dependent lipid efflux under basal conditions or following stimulation by cholesterol and LXR/RXR agonists was then performed. Loss of ABCA7 expression did not significantly affect efflux of cholesterol to apoA-I under basal or stimulated conditions (Fig. 6B). Because ABCG class transporters have recently been shown to mediate macrophages lipid efflux to mature HDL particles (24), we also assessed whether ABCA7 could mediate this function. As with apoA-I, the ABCA7 wild type and null macrophages effluxed similar amounts of sterol to mature HDL (Fig. 6C). Surprisingly, when apoA-I-dependent phospholipid efflux was measured under basal or stimulated conditions, no difference was detected between the wild type and null cells (Fig. 6D).

To clarify the unexpected finding that Abca7−/− macrophages effluxed similar amounts of phospholipids to apoA-I, we performed an in vitro experiment comparing phospholipid efflux in 293 cells transfected with either ABCA1 or ABCA7. Using ABCA1 and ABCA7 cDNA constructs that incorporated a FLAG epitope in the first extracellular loop, we measured efflux activity and normalized this value to cell surface trans-
Macrophage lipid efflux is unaffected by the loss of ABCA7 expression. A, bone marrow derived macrophages were assessed by immunoblotting for expression of ABCA7 (top) and ABCA1 (bottom) at baseline conditions and after stimulation with free cholesterol (10 μg/ml) and LXR/RXR agonists (10 μM 22-hydroxycholesterol and 10 μM 9-cis-retinoic acid) for 24 h. Under these conditions cholesterol efflux from wild type (WT) and null macrophages to apoA-I (10 μg/ml) for 20 h was measured as described under “Materials and Methods.” B, cholesterol efflux was determined as for C, substituting HDL (7.5 μg/ml) for apoA-I as the acceptor. D, phospholipid efflux from wild type and Abca7−−/− macrophages stimulated by apoA-I was measured under basal and stimulated conditions as described under “Materials and Methods.” E, phospholipid efflux from 293 or 293-EBNA-T cells transfected with either a FLAG-ABCA1 or a FLAG-ABCA7 construct was measured as described under “Materials and Methods.” Efflux values were then normalized to the cell surface expression level of the respective transporter as determined by anti-FLAG antibody binding to transfected cells. The assays were performed in triplicate with standard deviations depicted by error bars and are representative of two or more experiments. Ab, antibody.

**DISCUSSION**

Here we report the first analysis of mice lacking ABCA7 expression. ABCA7 is of considerable interest because it is a member of the A class of lipid transporters that have been shown to be causally implicated in three human genetic diseases: Tangier disease (ABCA1), Stargardt’s macular degeneration (ABCA4), and neonatal respiratory failure (ABCA3) (9-12,15). Lamellar ichthyosis type 2 maps to the ABCA12 locus in humans and may be a fourth human genetic disease caused by a member of this 12-gene family (25). Given the conservation of the A class transporters over millions of years of vertebrate evolution, it seems likely that many if not all of the family members will play a critical role in a physiologic process that involves lipid transport (22). ABCA7, in fact, has substantial sequence identity to ABCA1, and cell culture studies have demonstrated activity of the transporter in both phospholipid and cholesterol efflux to acceptor apoproteins (16, 17). Thus, our aim in inactivating ABCA7 in the mouse was to examine its impact on murine physiology and assess its importance in macrophage lipid efflux.

The results of our studies indicate that ABCA7 activity is not required for normal embryonic development and post-natal life. Extensive histologic examination of mouse tissues failed to reveal any obvious phenotypic abnormality. Abca7−−/− macrophages also displayed a normal capacity to efflux both phosphatidylcholine and cholesterol to apoA-I. This was true under basal conditions of transporter expression or following cholesterol loading and LXR/RXR nuclear hormone agonist activation, stimulations that are known to dramatically augment ABCA1 activity. Although lipid transport data from humans and mice lacking ABCA1 made it clear that ABCA7 is unable to compensate for the loss of ABCA1, our results indicate that ABCA1 cholesterol and phospholipid efflux activity does not depend on ABCA7 function. However, we did find lower HDL cholesterol levels in female Abca7−−/− mice. The mechanism of this gender-specific alteration in serum lipoproteins is currently being investigated in our lab, but our data provide little evidence to support a role for ABCA7 in sterol transport stimulated by apoA-I.

Because our data indicated that ABCA7 did not play an essential role in the efflux of cholesterol or phosphatidylcholine to apoA-I, we investigated other functions the transporter might have. Immunoblotting demonstrated a high level of expression in murine white adipose tissue, and cell culture experiments indicated that transporter expression was up-regulated during adipocyte differentiation. Fractionation of adipocytes indicated that ABCA7 partitioned in the microsomal and lipid droplet fractions, along with perilipin, a distribution distinctly different from that seen with ABCA1. Interestingly, the loss of ABCA7 expression in female mice was associated with a substantial reduction in fat mass. Despite these changes, ABCA7 expression was not required for maintaining circulating levels of triglycerides or free fatty acids.
Elucidating the functions of ABCA7 in fat is another area of active investigation in our laboratory. In addition to being highly expressed in white adipose tissue, ABCA7 is widely expressed in the brain. In situ hybridization analysis showed a clear neuronal localization of ABCA7 mRNA, particularly well seen in the neuron-dense hippocampal regions. Given the intense neuronal ABCA7 expression and mRNA, particularly well seen in the neuron-dense hippocampus, analysis showed a clear neuronal localization of ABCA7 in vivo. However, detailed studies of the behavior of Abca7−/− mice are currently in progress.

In summary we have found that ABCA7 is not required for murine embryonic development or post-natal survival. Although cell culture experiments had indicated a role in macrophage cholesterol and phospholipid trafficking when stimulated by apoA-I, Abca7−/− mice have no defect in apolipoprotein-stimulated sterol or phosphatidylcholine transport. Thus, we suggest that phosphatidylcholine and cholesterol are not likely to be the primary physiologic substrates of ABCA7 transporter activity. We further suggest ABCA7 may have a more specialized transport function, perhaps analogous to the role ABCA4 plays in the transport of N-retinylidene-phosphatidylethanolamine across membranes of the retina (6, 26). Further analysis of the Abca7−/− mice will be invaluable in determining the specific substrate transported by ABCA7 and its importance in vertebrate physiology.

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