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Comparison of the Physical Characteristics and Behavior in ABC Transporter A1, A7 or Apolipoprotein E Knockout Mice with Lipid Transport Dysfunction

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The physical characteristics and behavior of the ATP-binding cassette (ABC) A1, A7, and apolipoprotein (apo) E knockout (KO) mice with lipid transport dysfunction were investigated. These KO mice exhibited adequate growth, and their body masses increased steadily. No remarkable changes were observed in their blood pressure and heart rate. However, there was a slight increase in the heart rate of the ABCA7 KO mice compared with that of the wild-type (WT) mice. ABCA1 and apoE KO mice showed hypo- and hypercholesterol concentrations in the plasma, respectively. With regard to the cerebrum, however, the weight of the ABCA1 KO mice was lighter than those of the other genotypes. Furthermore, the cholesterol, triglyceride and phospholipid concentrations, and fatty acid composition were generally similar. Compared with the WT mice, ABCA1 KO mice stayed for a shorter time in the closed arm of the elevated plus maze, and performed worse in the initial stage of the Morris water maze. To thermal stimuli, the ABCA1 and apoE KO mice showed hyper- and hypo-sensitivities, respectively. Only the response of the ABCA1 KO mice was significantly inhibited by pretreatment with indomethacin. A low concentration of the prostaglandin E metabolites was detected in the plasma of the ABCA1 KO mice. Thus, ABCA1 is thought to play a specific role in the neural function.

Key words ATP-binding cassette (ABC) A1 transporter; ABCA7 transporter; apolipoprotein E; behavior; lipid

INTRODUCTION

ATP-binding cassette (ABC) transporters constitute one superfamily that is divided from seven subfamilies and involves 49 members in humans.^{1–3)} Of the ABC transporters, ABCA1 and ABCA7 are known to play important roles in lipid transport between cells and blood. Many reports indicate that ABCA1 carries cholesterol from cells to apolipoprotein A-I, and is essential to the formation of pre- β high-density lipoprotein (HDL) in the blood. Its genetic deficiency exists as Tangier disease in humans, which is characterized by low HDL concentration in the blood and excessive cholesterol accumulation in the cells.^{1–3)} These qualities have been observed in ABCA1 knockout (KO) mice.^{4,5)} ABCA1 is also involved in the absorption of cholesterol from food in the small intestine. With high homology to ABCA1, ABCA7 was shown to be involved in the transport of phospholipids and/or cholesterol in cultured cells.^{6–9)} However, ABCA7 KO mice do not exhibit outstanding abnormality of the lipid level in the plasma *in vivo*, and the reason for this is unclear.⁷⁾ Apolipoprotein E (apoE) is the main protein constituting lipoproteins, such as very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and HDL. After apoE is biosynthesized in the liver, it exists in forms bound to triglyceride (TG) and

cholesterol outside of the cells. These lipoproteins bind to the apoE receptor on the cell membrane, and the lipids are carried into cells. Therefore, apoE KO mice show hyperlipidemia, which produces atherosclerosis.^{10,11)}

Dysfunction of ABCA1, A7 or apoE changes the lipid concentration in the blood and affects various functions. First, this alters the release/biosynthesis of some bioactive substances, such as lipid mediators, cytokines, and steroid hormones.^{12–17)} Prostaglandins, one of the lipid mediators, are biosynthesized by phospholipase A₂ and cyclooxygenase from arachidonic acid in the cell membrane as needed, and are immediately released to the extracellular space. Therefore, these release/biosynthesis events will be influenced by the changed lipid compositions of the cell membrane. It has also been reported that ABCA1 and A7 are expressed on adipocytes and immune cells, and the dysfunctions change the cytokine release. Moreover, the decrease in cholesterol is linked to a reduction in the biosynthesis of steroid hormones. Second, the lipid metabolism is altered by the disturbed lipid homeostasis after the dysfunctions.^{1,5,8,18–24)} ABCA7 is reported to influence the expression of sterol regulatory element binding proteins, the transcription factors involved in the metabolism of fatty acids (FAs) and cholesterol, through changes in cholesterol homeostasis. Third, the dysfunction affects the cell membrane

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fluidity and/or caveola and raft structures after changes in the cell membrane lipid composition.^{25–28)} The fluidity and/or caveola and raft structures play an important role in determining the distribution of receptors, ion channels, and signaling molecules.^{25–28)} Finally, because lipids are needed to create a new cell membrane, lipids are essential for cell differentiation and proliferation, neurogenesis, synaptogenesis, and myelination.^{29–31)} The dysfunctions of ABCA1, ABCA7, and apoE probably produce problems in injury repair, memory formation, and other functions.

Recently, ABCA1, A7, and apoE have been reported to be expressed in the neurons and glial cells of the central nervous system (CNS), as well as the peripheral tissue.^{2,3,22,32,33)} Thus, these proteins may influence the regulation of CNS functions or lipid homeostasis. ABCA1, A7, and apoE are well-known risk factors of Alzheimer's disease, resulting from amyloid β (A β) processing.^{22,32–38)} Moreover, there is some evidence of apoE involvement in the pathogenesis of depression, epilepsy, or schizophrenia.^{32,37,39)} In this study, various types of behavior were compared using the three kinds of KO mice (ABCA1, A7, or apoE) with dysfunction of the lipid transport, and the influences of their differential lipid profiles on neural functions were investigated.

MATERIALS AND METHODS

Animals ABCA1 KO mice were provided by the Jackson Laboratory (Bar Harbor, ME, U.S.A.).⁴⁾ ABCA7 and apoE KO mice were provided by Prof. Mason W. Freeman (Lipid Metabolism Unit, Harvard Medical School, Boston, MA, U.S.A.)⁷⁾ and Prof. Nobuyo Maeda (Department of Pathology and Curriculum in Genetics, University of North Carolina (Chapel Hill, NC, U.S.A.),⁴⁰⁾ respectively. All KO mice were bred at the Animal Center of Nagoya City University Medical School and subsequently brought to Kinjo Gakuin University, where the experiments were carried out. Wild-type (WT) mice (C57BL/6J strain) were obtained from Japan SLC, Inc. (Shizuoka, Japan) as the control mice. These animals were housed at the Animal Center of Kinjo Gakuin University (maintained at 23 °C and 60% humidity with 12-h light period from 8:00 to 20:00), and given tap water and commercial normal food *ad libitum* for at least 2 weeks prior to the experiments. The 15–19 week-old male animals were divided into two groups; one for the open field test (OF) and hot plate test (HP), and another for the measurement of cardiovascular parameters, elevated plus maze test (EPM), and Morris water maze test (MWM). An interval of at least 10 d elapsed between the different behavior experiments. Several weeks after completion of the experiments, the animals were decapitated after anesthesia by sevoflurane to collect the plasma and cerebrum. In total, 28 WT mice, 23 ABCA1 KO mice, 20 ABCA7 KO mice, and 16 apoE KO mice were used in this study.

The experimental protocols were approved from the Animal Care and Use, and the Genetic Recombination Experiment Safety Committees of Kinjo Gakuin University.

Cardiovascular Parameter Mean blood pressure (MBP) and heart rate (HR) measurements were completed using a tail-cuff method. The mice were measured twice, several days apart, with a total of six measurements per animal. These averages were used as the experiment results. The measurements were carried out according to the manufacturer's instruction

using the attached software (MK-2000ST: Muromachi Kikai Co., Ltd., Tokyo, Japan).

Measurement of Lipid, Prostaglandin E (PGE) Metabolites, and Cytokine After decapitation with anesthesia by sevoflurane, blood was collected in an ice-cold tube with heparin. The plasma sample was the supernatant obtained by centrifugation (4 °C, 1600 $\times$ g, 15 min) of the blood. Samples were stored at –80 °C and used for the measurement of the total concentrations of cholesterol (TC), triglyceride (TG) and PGE metabolites (PGE M), and the cytokine array experiment. The cerebral hemisphere, obtained after decapitation, was also stored at –80 °C until measurement of TC, TG, phospholipid (PL) and FAs. The total lipid was extracted by the Bligh and Dyer method⁴¹⁾ after tissue sonication in phosphate buffered saline (PBS). The end product was dried under N₂ gas, and re-dissolved in methanol and chloroform. The lipid solution was divided into two aliquots to measure the TC, TG, and PL concentrations, and the composition of FAs. TC, TG, and PL concentrations were measured by the colorimetric method.⁴²⁾ The method of the FA measurement was previously described elsewhere.⁴³⁾ In brief, after drying the prepared lipid solution, it was again dissolved in methanol and methylated by heat. The methylated FAs were then extracted by petroleum ether, and various FAs were measured by GLC (Shimadzu, Kyoto, Japan). FA 17:0 was added before the extraction procedure as an internal standard. For PGE M measurements and the cytokine array experiment, commercial kits were used (Prostaglandin E Metabolite ELISA Kit: Cayman Chemical Company, MI, U.S.A., and Proteome profiler Array, Mouse Cytokine Array Panel A: R&D Systems, Inc., MN, U.S.A.), and the procedures were performed according to the manuals of the two respective kits. For the cytokine array, equal plasma volumes from four mice were mixed for each genotype, and subsequently run on the array membrane. ImageJ software (National Institutes of Health, Bethesda, MD, U.S.A.) was used to analyze the membrane spots.

Behavioral Experiments The animals were acclimated to the testing room and experimental handling for behavioral experiments for a few days before the experiments. On the experimental day, the animals were brought into the room 1–2 h before the experiments. The experiments were performed from 11:00 a.m. to 03:00 p.m. The apparatus was kept in the same place, and was wiped with ethanol after each test. During experiments, the operators were careful to minimize being seen by the subject. Each behavioral experiment was carried out at least 10 d apart.

OF tests were carried out using a 150-cm-diameter circular pool with a 50-cm-high wall to measure the locomotor activities. Mice entered the field from the same point and were observed for 60 min. The analyses were carried out using video tracking software (ANY maze; Stoeling Co., IL, U.S.A.).

The instrument for EPM was composed of four arms (5 cm wide, 30 cm long and 90 cm high from the floor); two closed arms and two open arms with and without a 25-cm-high wall, respectively. At the start, the animal was put in the center zone (5 $\times$ 5 cm) toward the open arm. When the mouse was placed in the open arm, it was counted as the entry number. The behaviors of the mice were observed for 5 min. The anxiety-related behavior was analyzed by the amount of time spent in the open and closed arms using the video tracking system.

MWM was carried out using the pool for OF (a 150-cm-

diameter pool) with 20-cm-depth water at 23–25°C. The island (a 10-cm-diameter cylinder made from clear plastic) was set in the center of one of the four divided pools at 1 cm under the water surface. Mice entered the pool from the farthest point from the island, and were forced to swim for 60 s. When the mice found an island in the pool, it was possible for them to rest on it. The time was measured from the initiation of swimming to reaching the island using the video tracking system. MMW was conducted three times in the morning and afternoon each day for 6 consecutive days for all mice. For mice unable to find the island within 60 s, they were placed on the island for 15 s, and the data in this case were calculated as 60 s. After the test, the mice were dried with a towel. The recording and analysis were carried out using the video tracking system.

Responses to heat were measured as the elapsed time before jumping up from a hot plate (HP) set at $55 \pm 0.2^\circ\text{C}$ using a hot plate instrument (KN-205; Natsume Seisakusho Co., Ltd., Tokyo, Japan). Mice were first acclimated several times before measurements were started. A cutoff time was set 15 and 50 s before and after the drug injection, respectively. The average of two measured values was used as the experimental data.

Statistical Analysis All data were expressed as the mean $\pm$ standard error (S.E.). Statistical analysis was carried out using one-way or two-way ANOVA, followed by the Tukey *post-hoc* test. Differences were considered significant when the *p* value was <0.05 .

Drugs Indomethacin was obtained from Sigma-Aldrich Inc. (MO, U.S.A.) and buprenorphine (Lepetan[®]) was obtained from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). All other reagents used were of high grade. Indomethacin was dissolved in 0.1 N NaOH and diluted 20-fold with PBS. The other drugs were dissolved in sterile physiological saline.

RESULTS

Physical Characteristics At the beginning (at 15–19 weeks old) and/or end (at 25–30 weeks old) of the experiments, the body weight, cerebral weight, MBP, and HR of the WT mice and ABCA1, ABCA7, and apoE KO mice were measured. As shown in Table 1, the growth of the three KO mice was comparable to that of WT mice by body weight comparison. The MBP values were similar among the WT mice and the other KO mice (WT: 88.2 ± 2.9 mmHg, *N* = 10; ABCA1 KO: 89.0 ± 1.6 mmHg, *N* = 8; ABCA7 KO: 84.9 ± 3.9 mmHg, *N* = 8; apoE KO: 86.7 ± 3.2 mmHg, *N* = 8). Compared with the other mice, the ABCA7 KO mice showed a slightly higher HR, and this difference was statistically significant (WT: 689.3 ± 8.6 beats/min, *N* = 10; ABCA1 KO: 700.2 ± 4.2 beats/min, *N* = 8; ABCA7 KO: $737.1 \pm 9.1^*$ beats/min, *N* = 8; apoE KO: 701.3 ± 9.3 beats/min, *N* = 8; $*p < 0.05$ vs. the other groups). These three types of KO mice generally grew steadily. However, when comparing the cerebral weight of the four genotypes of mice, the ABCA1 KO mice exhibited significantly lighter cerebral weight than the other KO mice and WT mice (Table 1).

Because ABCA1, ABCA7, and apoE are involved in lipid transport/metabolism, plasma concentrations of TC and TG in their KO mice were measured (Table 2A). ABCA1 and apoE KO mice showed severely hypo- and hyper-TC concentrations in the plasma, respectively. Conversely, the TC concentration in the plasma of ABCA7 KO mice was not significantly different from that of WT mice. The TG concentrations of the other mice genotypes did not significantly differ. The TG concentration in the plasma of the ABCA1 KO mice was slightly lower than that of WT mice, but the decrease was not statistically significant. These concentrations and the FA composi-

Table 1. Body and Cerebral Weight of WT, A1 KO, A7 KO and apoE KO Mice

	WT	A1 KO	A7 KO	apoE KO
Body weight (g, 15–19 week-old)	28.4 ± 0.6	25.7 ± 0.5	27.5 ± 0.5	28.3 ± 1.1
Body weight (g, 25–30 week-old)	33.2 ± 1.0	33.0 ± 0.9	33.1 ± 0.9	30.5 ± 1.0
Cerebral weight (mg, 25–30 week-old)	320.4 ± 10.7	$275.5 \pm 3.7^*$	332.3 ± 6.6	313 ± 4.1

Body weight (15–19 week-old) WT: *N* = 15; ABCA1 KO: *N* = 17; ABCA7 KO: *N* = 16; apoE KO: *N* = 9. Body weight (25–30 week-old) WT: *N* = 28; ABCA1 KO: *N* = 23; ABCA7 KO: *N* = 20; apoE KO: *N* = 16. Cerebral weight WT: *N* = 10; ABCA1 KO: *N* = 10; ABCA7 KO: *N* = 11; apoE KO: *N* = 10. $*p < 0.05$ vs. WT: One-way ANOVA following Tukey *post-hoc* test.

Table 2. Concentration of Cholesterol (TC) and Triglyceride (TG) in the Plasma (A) and Cerebrum (B)

(A) Plasma				
	WT	A1 KO	A7 KO	apoE KO
TC	71.3 ± 5.7	$6.2 \pm 0.9^*$	74.3 ± 6.1	$648.4 \pm 56.8^*$
TG	30.5 ± 5.2	19.0 ± 5.1	34.4 ± 8.5	38.0 ± 11.1
WT: <i>N</i> = 6; ABCA1 KO: <i>N</i> = 6; ABCA7 KO: <i>N</i> = 7; apoE KO: <i>N</i> = 5. $*p < 0.05$ vs. WT mice; mg/dL.				
(B) Cerebrum				
	WT	A1 KO	A7 KO	apoE KO
TC	17.3 ± 1.8	14.1 ± 0.4	14.0 ± 0.4	17.5 ± 1.5
TG	1.6 ± 0.2	2.0 ± 0.1	1.7 ± 0.2	1.7 ± 0.4
PL	24.2 ± 1.6	21.8 ± 0.3	22.8 ± 0.4	21.7 ± 0.4

N = 5; $\mu\text{g}/\text{mg}$ wet weight.

Table 3. Composition of FAs in the Cerebrum

	WT	A1 KO	A7 KO	apoE KO
16:0 DMA	1.28 ± 0.10	1.08 ± 0.15	1.17 ± 0.08	0.00 ± 0.00
16:0	16.84 ± 0.91	17.89 ± 0.33	15.89 ± 1.04	5.43 ± 0.91*
16:1	0.23 ± 0.12	0.36 ± 0.06	0.36 ± 0.04	0.00 ± 0.00
18:0 DMA	3.30 ± 0.46	3.15 ± 0.35	3.45 ± 0.11	14.44 ± 0.93*
18:0	28.48 ± 3.72	23.23 ± 0.44	21.36 ± 1.23	20.23 ± 1.33
18:1 DMA	2.33 ± 0.34	1.45 ± 0.12	1.45 ± 0.06	3.35 ± 0.81
18:1 n-9	12.98 ± 1.90	15.15 ± 0.21	14.52 ± 0.83	10.54 ± 0.79
18:1 n-7	2.72 ± 0.29	3.25 ± 0.02	3.05 ± 0.18	2.44 ± 0.19
18:2 n-6	1.68 ± 0.30	0.54 ± 0.14	0.61 ± 0.05	2.11 ± 0.42
18:3 n-6	0.13 ± 0.08	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.02
18:3 n-3	0.06 ± 0.02	0.04 ± 0.00	0.04 ± 0.01	0.06 ± 0.02
20:0	0.46 ± 0.15	0.02 ± 0.00	0.03 ± 0.01	0.60 ± 0.05
20:1	1.41 ± 0.13	1.62 ± 0.06	1.66 ± 0.09	1.56 ± 0.10
20:2 n-6	0.15 ± 0.08	0.12 ± 0.01	0.11 ± 0.01	0.15 ± 0.01
20:3 n-6	0.48 ± 0.05	1.25 ± 0.19	2.06 ± 0.74*	0.56 ± 0.04
20:4 n-6	8.44 ± 0.75	9.84 ± 0.23	9.95 ± 0.05	9.22 ± 0.61
20:3 n-3	0.02 ± 0.01	0.05 ± 0.00	0.05 ± 0.00	0.13 ± 0.08
20:5 n-3	0.03 ± 0.02	0.01 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
22:0	0.95 ± 0.26	0.56 ± 0.22	0.98 ± 0.24	1.43 ± 0.16
22:1	0.21 ± 0.16	0.64 ± 0.54	1.02 ± 0.30	0.56 ± 0.36
22:2	0.14 ± 0.10	0.01 ± 0.01	0.00 ± 0.00	0.07 ± 0.03
22:4 n-6	1.67 ± 0.93	0.34 ± 0.09	0.39 ± 0.01	4.44 ± 0.30*
22:5 n-3	0.20 ± 0.05	0.13 ± 0.01	0.11 ± 0.03	0.15 ± 0.05
22:6 n-3	15.43 ± 1.27	16.14 ± 0.42	15.45 ± 0.56	18.64 ± 1.30
24:0	1.43 ± 0.51	0.76 ± 0.06	0.70 ± 0.06	1.91 ± 0.49
24:1	2.16 ± 0.12	2.29 ± 0.07	2.08 ± 0.10	3.63 ± 0.50*
n-6/n-3 ratio	0.81 ± 0.08	0.76 ± 0.04	0.72 ± 0.05	0.87 ± 0.02
Saturated FA	49.49 ± 3.23	48.71 ± 2.03	43.57 ± 2.43	42.32 ± 3.23
Monoene FA	22.05 ± 2.04	24.37 ± 1.23	23.94 ± 1.41	22.09 ± 0.96
n-6 FA	12.58 ± 1.41	11.65 ± 0.53	13.16 ± 0.67	16.55 ± 1.22
n-3 FA	15.74 ± 1.25	15.25 ± 0.81	19.32 ± 3.08	18.98 ± 1.32
Other	0.14 ± 0.10	0.02 ± 0.02	0.00 ± 0.00	0.07 ± 0.03

Values of FAs represent mean ± S.E. of % of total FA ($\mu\text{g}/\text{mg}$ of tissue). $N=5$; * $p<0.05$ vs. WT mice. DMA: dimethylacetal derivatives.

tion in the cerebrum were investigated for these genotypes. As shown in Table 2B, the WT and KO mice showed similar TG, TC, and PL concentrations. For the FA concentration in the cerebrum (Table 3), some FA concentrations of the KO mice (20:3 n-6 in ABCA7 KO mice and 16:0 dimethylacetal derivatives (DMA), 16:0, 18:0 DMA, 22:4 n-6 and 24:1 in apoE KO mice) significantly changed from that in the WT mice. However, the FA compositions of the four types of mice were only slightly different. The ratio of n-6/n-3, saturated FA, and monoene FA in the KO mice were similar to those of WT mice.

Behavior Using OF, the locomotor activities were investigated for all genotypes. The traveling distance and staying time in the side zone (20-cm-wide zone from the wall of the pool) for 0–60 min were not different among the four kinds of mice (Fig. 1). The ABCA7 KO mice seemed to show slightly less movement than the other genotypes. The difference was statistically significant compared with the ABCA1 KO mice, but not with the WT mice. These indices for 15 min interval were not statistically different from those of the WT mice.

Figure 2 shows the results in the EPM experiment. WT mice did not stay in the open arm, and spent most of the experimental time in the closed arm. In addition, the behavior of the ABCA7 and apoE KO mice was similar to that of WT

Fig. 1. Locomotor Activity in the Open Field

The total traveling distance and the time spent in the side zone for 0–60 min of the open field experiment are shown. The side zone was set up as the 20-cm-wide space from the wall to the inside. There was no significant difference compared with WT mice. WT: $N=18$; ABCA1 KO: $N=14$; ABCA7 KO: $N=11$; apoE KO: $N=8$.

mice. ABCA1 KO mice, like the other genotypes, preferred the enclosed arm to the open arm. However, their staying time in the closed arm was significantly less than that of the other genotypes. ABCA1 KO mice spend more time in the center zone, compared with the other genotypes. Although the ABCA1 KO mice went into the closed arm, they frequently returned to the center zone and remained there. There was no statistical significance in the differences among the total

Fig. 2. Behavior in the Elevated Plus Maze

The times spent in the open arm zone (A), in the enclosed zone (B), and in the central zone (C) of the elevated plus maze are shown. The mice were put in the central zone (5 × 5 cm square) at the start time-point of the test. The ABCA1 KO mice preferred to stay in the central zone significantly more than the enclosed zone, compared with the other mice. The total traveling distances of the WT mice and the three KO mice were not significantly different (data not shown). * $p < 0.05$ vs. WT mice. WT: $N = 10$; ABCA1 KO: $N = 10$; ABCA7 KO: $N = 9$; apoE KO: $N = 9$.

Fig. 3. Latency to Reach the Island in the Morris Water Maze

The first number of the “Stage” shows the day number of the experiment. The second number (“1” or “2”) shows whether the tests were carried out in the morning or afternoon of the day of the first number, respectively. In the afternoon trial of the first day, the WT mice already comparatively memorized the island site of the water maze. However, all KO mice displayed poor performances. On the fifth day, the ABCA7 and apoE KO mice needed longer times than the other mice to reach the island. * $p < 0.05$ vs. WT mice at the same time-point. WT: $N = 10$; ABCA1 KO: $N = 9$; ABCA7 KO: $N = 9$; apoE KO: $N = 8$.

traveling distances (WT: 5.70 ± 0.70 m, $N = 10$; ABCA1 KO: 5.67 ± 0.81 m, $N = 10$; ABCA7 KO: 4.51 ± 0.50 m, $N = 9$; apoE KO: 5.23 ± 0.64 m, $N = 9$) or entry numbers to the open arm (WT: 1.33 ± 0.56 counts, $N = 10$; ABCA1 KO: 3.67 ± 0.75 counts, $N = 10$; ABCA7 KO: 1.00 ± 0.44 counts, $N = 9$; apoE KO: 2.00 ± 0.67 counts, $N = 9$) of the four groups.

The cognitive function was investigated in MWM (Fig. 3). At Stage 1–2 of this study, which was the trial carried out on the afternoon of the first day, the WT mice remembered the island position in the pool well. Thereafter, the time required for the WT mice to reach the island gradually decreased. On the fifth day, the mice reached the island in approximately 7 s. In contrast, the three types of KO mice took longer than the WT mice to reach the island at Stage 1–2. Of the KO mice, the ABCA1 mice caught up with the WT mice, and their performance was similar to that of the WT mice on the fifth day. In contrast, although the abilities of the ABCA7 and apoE KO mice gradually improved, their times to reach the island on the fifth day were still longer than that of the WT mice.

As shown in Fig. 4, the ABCA1 KO mice showed increased

sensitivity to thermal stimuli compared with the WT mice. Conversely, the apoE KO mice showed less sensitivity, and the ABCA7 KO mice showed normal sensitivity (Fig. 4A). The pretreatment with indomethacin (10 mg/kg, intraperitoneally (i.p.)) significantly lengthened the reaction time in only ABCA1 KO mice in comparison with the vehicle-treated mice in each genotype group, and the sensitivities were similar among the four genotypes. The pretreatment with buprenorphine (2.0 mg/kg, i.p.), a partial opioid agonist, inhibited the response in all mice in comparison with the vehicle-treated mice of the same genotype. However, the threshold time to the response in the ABCA1 and ABCA7 KO mice was evidently shortened in comparison with the buprenorphine-treated WT mice.

As described above, indomethacin-induced inhibition was observed in the ABCA1 KO mice. Therefore, the PGE M concentration in the plasma was measured. In contrast to expectations, the PGE M concentration in the ABCA1 KO mice was significantly lower than that of the WT mice (Fig. 5). The ABCA7 KO mice showed a PGE M concentration that was only slightly lower than that of the WT mice. The apoE KO mice had a normal PGE M concentration. In addition, the measurement of the inflammation-related cytokine concentration in the plasma was carried out using the array kit (Fig. 6). Overall, the cytokine concentrations in the ABCA1 and ABCA7 KO mice were nearly identical to that of WT mice. However, the concentrations of many cytokines in the apoE KO mice were generally lower than that of WT mice. In addition, some interleukins (IL-1 β , -1 receptor antagonist, -2, -5, -6, -17, -23, -27), macrophage inflammatory proteins (MIP)-1 β , -2, regulated on activation, normal T-cell-expressed and secreted chemokine (RANTES), the stromal cell-derived factor (SDF), and thymus and activation-regulated chemokine (TARC) could not be detected in the apoE KO mice.

DISCUSSION

The ABCA1, ABCA7, and apoE KO mice used in this study steadily gained body mass, and their MBP and HR were generally similar to that of the WT mice. However, the ABCA7 KO mice exhibited slightly increased HR. Concerning the TC

Fig. 4. Threshold Time to Thermal Stimuli in the Hot Plate

The data present the threshold time under the condition with no treatment (A), and at 45 min after ip injection of the vehicle, indomethacin (10 mg/kg) or buprenorphine (2.0 mg/kg) (B). The ABCA1 and apoE KO mice showed hyper- and hypo-sensitivity, respectively. The pretreatment with indomethacin only inhibited the response of the ABCA1 KO mice, and produced similar responses in the four mice groups. Conversely, inhibition by the pretreatment with buprenorphine were observed in all mice groups. However, the inhibitory effects of the WT and apoE KO mice were stronger than those of the other groups. * $p < 0.05$ vs. WT mice in each drug-treated group. # $p < 0.05$ vs. saline-treated mice in each genotype groups. WT: $N = 18$; ABCA1 KO: $N = 15$; ABCA7 KO: $N = 9$; apoE KO: $N = 7$.

concentration in the plasma, the ABCA1 and apoE KO mice showed extremely low and high concentrations, respectively, compared with the WT mice. The TC concentration in the ABCA7 KO mice was not significantly different from that of WT mice. In contrast, the TG concentrations in the plasma were not statistically different from each other, although the ABCA1 KO mice tended to show low TG concentrations compared with the other groups. These results are consistent with previous reports.^{44,45)} ABCA7 was demonstrated to transport phospholipids and/or cholesterol in cultured cells. However, no obvious abnormalities of the TC or TG concentration were found in the plasma from ABCA7 KO mice.⁶⁻⁹⁾ It is unclear why no abnormalities were found, but the other transporters might compensate functionally when ABCA7 does not work. Alternatively, ABCA7 participates in the other functions *in vivo*, but not lipid transport. Recently, the roles of ABCA7 in phagocytosis or Alzheimer's disease have attracted much interest.^{24,33,36,46)}

The TC, TG, and PL concentrations in the cerebrum were not statistically different among the three types of KO mice and the WT mice. In addition, their FA compositions in the cerebrum were not noticeably different. The CNS maintained relative lipid homeostasis in the KO mice, even if there were abnormalities in the cholesterol concentration in the plasma. It was demonstrated that the neurons and/or glial cells in the CNS have various enzymes for cholesterol biosynthesis.^{20,29-31)} Despite having similar body weight and lipid composition in the CNS, the cerebral weight of the ABCA1 KO mice was significantly lighter than that of the other mouse genotypes. ABCA1 is expressed in the reproductive organs, such as the placenta, myometrium, and fetal membranes.^{12,47)} The ABCA1 deficiency in the reproductive organs, in addition to the extremely low concentration of TC, progesterone or estrogen in the mother's plasma, may result in poor embryo growth. During the fetal period, lipids are a critical nutrient and an appropriate concentration is needed. In fact, pups of ABCA1 KO mice tend to be born small. Although the body weight of ABCA1 KO mice gradually catches up to that of WT mice after birth, their cerebrum mass still remains lower even when they reach maturity. Karasinska *et al.*⁴⁸⁾ generated brain-spe-

Fig. 5. Concentration of Prostaglandin E Metabolites in the Plasma

The ABCA1 KO mice demonstrated significantly low PGE M concentration in the plasma. Its concentration in the ABCA7 KO mice was only slightly decreased. * $p < 0.05$ vs. WT mice. $N = 5$.

cific ABCA1 KO mice, and demonstrate that their cholesterol uptake from the peripheral system to the CNS increases to compensate for the lack of cholesterol. The cholesterol concentration and number of synapses in the brain cortex of the mice then decrease, but those in the hippocampus do not. The cerebral mass and body weight of brain-specific ABCA1 KO mice are not shown. In this study, the TC concentration in the whole cerebrum of the ABCA1 KO mice was not statistically different from that of WT mice. Concerning the behavior during the OF experiment, there are differences between the two types of ABCA1 KO mice. There were decreased and similar locomotor activities of the brain-specific ABCA1 mice and our whole ABCA1 KO mice, respectively. One reason for these discrepancies may be the differences of the deficient condition, which brings different compensatory reactions depending on the situation of each lipid concentration. In addition, our MWM experiments showed that the performances of the ABCA1 KO mice were inferior during stage 1-2 in the afternoon on the first experimental day. This may indicate that their initial memory is inferior. Their performances seemed to improve with repeated practice. This result suggested that ABCA1 plays some role in memory ability in the CNS.

Fig. 6. Detection of Various Cytokines in the Plasma

The detection of various cytokines in the plasma was carried out using a cytokine array kit. The array membrane was reacted with the plasma collected from four mice in each genotype group. The cytokine concentrations in the apoE KO mice were generally lower than that of the other groups. BLC (CXCL13/BCA-1), B lymphocyte chemoattractant; C5/C5a, complement component 5/5a; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; I-309 (CC ligand 1/TCA-3), T lymphocyte-secreted protein; sICAM-1 (CD54), soluble intercellular adhesion molecule-1; IFN- γ , interferon; IL, interleukin; IP-10 (CXCL10/CRG-2), interferon γ -induced protein 10; I-TAC (CXCL11), interferon-inducible T-cell α chemoattractant; KC (CXCL1), keratinocyte-derived chemokine; M-CSF, macrophage colony-stimulating factor; MCP-1 (CCL2), monocyte chemoattractant protein-1; MCP-5 (CCL12), monocyte chemoattractant protein-5; MIG (CXCL9), monokine induced by gamma interferon; MIP, macrophage inflammatory proteins; RANTES (CC ligand 5), regulated on activation normal T cell expressed and secreted; SDF, the stromal cell-derived factor; TARC (CC ligand 17), thymus and activation-regulated chemokine; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor; TREM, triggering receptor expressed on myeloid cell.

ABCA1 KO mice also demonstrated increased and decreased staying times in the central zone and the closed arm zone in our ERM experiments, respectively. Although EPM is generally used to test for anxiety behavior, our result was not a typical attenuation of anxiety behavior. We supposed that this may reflect a certain lack of cognitive function, and serves as evidence for ABCA1 in playing some role in cognitive function. ABCA1 KO mice show different patterns of cognitive deficit from the ABCA7 or apoE KO mice described below. ABCA1 KO mice with or without administration of the A β precursor protein show cognitive deficits in MWM or in novel object recognition experiments.^{49,50} ABCA1 is recognized to be a risk factor for Alzheimer's disease because of A β accumulation through changes in the lipidation state of apoE, raft structure and/or neural structure.³⁸ In the ABCA7 and apoE KO mice, inferior performances in both early and late stages of the MWM/novel object recognition task and normal behavior in the EPM experiment have been shown.^{11,24,51} This is consistent with our results. To date, many studies in the literature indicate that ABCA7 and apoE deteriorations are risk factors for Alzheimer's disease.^{32–37} Concerning the roles of ABCA7 in Alzheimer's disease, some studies in the literature have reported increases in the A β processing/accumulation in ABCA7-deficient situations, and the increased incidence of Alzheimer's disease in humans having a premature codon of ABCA7.^{24,33,36,46} In contrast, the involvement of apoE with Alzheimer's disease is diverse.^{17,32,34,35,37,39,52} Of the apoE1–4 alleles in humans, apoE4 binds the most strongly to A β . Following decreased degradation of A β , however, the binding is dependent on the lipidation state of apoE4. ApoE regulates the processing, clearance, and degradation of A β . It has also been suggested apoE deficiency possibly produces atherosclerosis in the brain, and this plays some role in vascular cognitive impairment.^{11,34} Thus, apoE is involved in the progression of Alzheimer's disease in various aspects, and is recognized as one of the strongest risk factors.

The ABCA1 and apoE KO mice showed hyper- and hypo-sensitivities to thermal stimuli, respectively. In the ABCA7 KO mice, the sensitivity was nearly identical to that in the

WT mice. Only the response of the ABCA1 KO mice was inhibited by the ip pretreatment with indomethacin. Therefore, PGs derived from the peripheral tissue are probably involved in the observed hypersensitivity. However, the ABCA1 KO mice showed the lowest PGE M concentration in the plasma among the four genotypes. PGs are well known to biosynthesize from arachidonic acid in the cell membrane and to produce pain.⁵³ The arachidonic acid concentration may be abnormal in the plasma membrane of the peripheral tissue of ABCA1 KO mice, although this has not yet been determined. ABCA1 may also be required for expression of the PG synthesis enzymes. We suppose that this PGEM decrease resulted in the upregulation of the EP receptor in the peripheral system. The slight increase in PGE₂ after thermal stimuli then produces the hyper-response. Conversely, the hyposensitivity of the apoE KO mice was similar to that of previous reports.^{54,55} ApoE KO mice were demonstrated to produce irregular forms and decreased number of neurons, and an impaired intracellular signaling pathway of the nerve growth factor receptor. We suppose that the decrease in cytokines is partially responsible for the hyposensitivity because the cytokine concentrations that influence the sensory nerve activity to noxious stimuli were extremely low in apoE KO mice, as shown in this study and previous studies.^{54,55} Many cytokines influence pain sensation.^{56–59} In addition, similar hypoalgesia has been reported under the condition of decreased cytokine concentration in the plasma after the administration of statins.^{60,61} Statins are well known to block cholesterol synthesis by inhibition of hydroxymethylglutaryl-CoA (HMG-CoA) reductase. The pretreatment with buprenorphine inhibited the response in all mice. However, the inhibition in ABCA1 and ABCA7 KO mice was significantly weaker than in WT mice, although the dose used was enough to produce the maximum effects.^{62,63} The sensitivity to thermal stimuli was promoted in ABCA1 KO mice, which is a possible cause of the weak inhibition. In contrast, the inhibition was weak in ABCA7 KO mice despite the sensitivity being similar to that of WT mice. The main action sites of analgesia induced by buprenorphine are μ -opioid receptors on the ascending neurons for nociceptive informa-

tion to the CNS, and the descending neurons for pain control from the mid brain and medulla to the spinal cord. Therefore, ABCA7 KO mice may have some damage in the descending neuron, compared with the ascending neurons.

In summary, ABCA1 KO mice demonstrated a very early stage of cognitive deficit and hyper-analgesia because of an underdeveloped CNS and/or low PG level in the plasma. In ABCA7 and apoE KO mice, cognitive deficit and combined cognitive deficit with hypo-analgesia, respectively, were observed.

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Conflict of Interest The authors declare no conflict of interest.

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