PCN Psychiatry and Clinical Neurosciences

REGULAR ARTICLE

Cholesterol profiling reveals 7β-hydroxycholesterol as a pathologically relevant peripheral biomarker of Alzheimer's disease

Junghee Ha, MD, PhD [1], Go Eun Kwon, MS [2], Yumi Son, BS [1,3], Soo Ah Jang, MD, PhD [1], So Yeon Cho, PhD [1,3], Soo Jin Park, PhD [4], Hyunjeong Kim, PhD [1,5], Jimin Lee, MS [1], Juseok Lee, MD [6], Dongryul Seo [6], Myeongjee Lee, PhD [7], Do Yup Lee, PhD [4], Man Ho Choi, PhD [2]* and Eosu Kim, MD, PhD [1,3,5]*

Aim: Cholesterol homeostasis is associated with Alzheimer's disease (AD). Despite the multitude of cholesterol metabolites, little is known about which metabolites are directly involved in AD pathogenesis and can serve as its potential biomarkers.

Methods: To identify "hit" metabolites, steroid profiling was conducted in mice with different age, diet, and genotype and also in humans with normal cognition, mild cognitive impairment, and AD using gas chromatography–mass spectrometry. Then, using one of the "hit" molecules (7β-hydroxycholesterol; OHC), molecular and histopathological experiment and behavioral testing were conducted in normal mice following its intracranial stereotaxic injection to see whether this molecule drives AD pathogenesis and causes cognitive impairment.

Results: The serum levels of several metabolites, including 7β-OHC, were increased by aging in the 3xTg-AD unlike normal mice. Consistently, the levels of 7β-OHC were increased in the hairs of patients with AD and were correlated with clinical severity. We found that 7β-OHC directly affects AD-related pathophysiology; intrahippocampal injection of 7β-OHC induced astrocyte and microglial cell activation, increased the levels of pro-inflammatory cytokines (TNF-alpha, IL-1β, IL-6), and enhanced amyloidogenic pathway. Mice treated with 7β-OHC also exhibited deficits in memory and frontal/executive functions assessed by object recognition and 5-choice serial reaction time task, respectively.

Conclusions: Our results suggest that 7β-OHC could serve as a convenient, peripheral biomarker of AD. As directly involved in AD pathogenesis, 7β-OHC assay may help actualize personalized medicine in a way to identify an at-risk subgroup as a candidate population for statin-based AD treatment.

Keywords: *7β-hydroxycholesterol, Alzheimer's disease, biomarker, cholesterol metabolites, neuroinflammation.*

http://onlinelibrary.wiley.com/doi/10.1111/pcn.13706/full

Cholesterol plays a pivotal role in the development of Alzheimer's disease (AD).[1,2] Elevated cholesterol levels correlated with enhanced production of β-amyloid (Aβ) in neuronal cells,[3] and a high-fat and high-cholesterol (HFC) diet was found to induce significant cognitive dysfunction in rodents.[4] Epidemiological studies have also revealed a close link between high levels of cholesterol in midlife and the increased incidence of AD later in life.[5,6] However, the whole picture seems to be more complicated than what is suggested by the findings of these previous studies. For instance, a large population-based cohort study showed no association between total cholesterol levels and the risk of AD.[7] Furthermore, lower, not higher, cholesterol levels are associated with worsening cognitive function in old age,[8] while cholesterol esters rather than free cholesterol may contribute to AD pathogenesis.[9] A recent study using AD-patient isogenic induced pluripotent stem cell (iPSC)-derived neurons also proposed that cholesteryl esters can independently regulate Tau and Aβ.[10] One of the reasons for these conflicting findings in human study could be that total cholesterol levels cannot reflect the diversity and complexity of cholesterol metabolism.

Cholesterol is metabolized into physiologically active sterols, and some of them, such as cortisol and estrogen, are involved in learning and memory in addition to their traditional functions.[11,12] Oxysterols have been particularly implicated in the pathogenesis of AD and elevated levels of 24S-hydroxycholesterol (24-OHC), 27-OHC, and 7β-OHC have been observed in the plasma, cerebrospinal fluid, and brain tissues of patients with AD.[13–15] Despite the relevance and complexity of cholesterol biosynthesis and metabolism concerning AD pathogenesis, limited studies have explored how the

[1] Department of Psychiatry, Laboratory for Alzheimer's Molecular Psychiatry, Institute of Behavioral Science in Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea
[2] Molecular Recognition Research Center, Korea Institute of Science and Technology, Seoul, Republic of Korea
[3] Graduate School of Medical Science, Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea
[4] Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Research Institute for Agricultural and Life Sciences, Seoul National University, Seoul, Republic of Korea
[5] Metabolism-Dementia Research Institute, Yonsei University College of Medicine, Seoul, Republic of Korea
[6] Department of Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea
[7] Biostatistics Collaboration Unit, Department of Biomedical Systems Informatics, Yonsei University College of Medicine, Seoul, Korea
* Correspondence: Email: mh_choi@kist.re.kr and eosu.kim@yonsei.ac.kr

metabolic signatures of cholesterol are altered by several risk factors for AD, such as aging and metabolic stress.

Therefore, the objectives of this study were twofold. First, we aimed to identify cholesterol precursors and metabolites whose levels change with aging in 3xTg-AD mice, a transgenic AD model. We explored whether the levels of these sterols in human hair are associated with the diagnosis and clinical status of AD. Second, we aimed to conduct *in vivo* studies to assess whether a "hit" metabolite (7β-OHC in this study) has specific effects on AD pathology and behavior.

Methods

Participants

Participants were categorized into three groups: normal cognition (NC), mild cognitive impairment (MCI), and AD based on their cognitive status. We recruited a cohort of 285 participants sourced from a university-affiliated hospital and local dementia centers from 2013 to 2015 (Fig. S1). The participant demographics are displayed in Table 1. The study was approved by the Institutional Review Board at Yonsei University Health System (2013-1740-004) and was conducted in accordance with the Helsinki Declaration. All subjects provided written informed consent. Participants aged 60–80 years with a Global Deterioration Scale (GDS) score ≤5 were included. Individuals were excluded if they had (1) significant psychiatric disorders; (2) previous major neurological illness such as stroke, traumatic brain injury, or epilepsy; (3) prior diagnoses of other forms of dementia such as vascular dementia, Lewy body disease, or frontotemporal dementia; (4) a GDS score exceeding 5. All participants were assessed by two board-certified psychiatrists, who conducted clinical interviews and performed neuropsychological tests. Diagnostic classification was based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) for dementia of Alzheimer's type[16] and Peterson's criteria for MCI.[17]

Quantification of cholesterol metabolites

Quantitative metabolite profiling of humans and mice was performed based on a validated method as previously described.[18–20] A detailed description of cholesterol profiling can be found in the Supplementary Data.

Animals and treatment

In the first part of the animal study, in which steroid profiling was performed, 24 triple transgenic mice carrying the familial AD mutations APPSwe, PS1/M146V, and tauP301L, known as 3xTg-AD mice (The Jackson Laboratory, Bar Harbor, ME) and 24 wild-type C57BL6 mice (Animal Facility of Aging Science, Gwangju, South Korea) were used. Two different age groups were included for each mouse strain: the young group (12 weeks old, $n = 12$) and old group (44–48 weeks old, $n = 12$). The mice were randomly distributed into two groups according to diet: the normal diet (ND) group (11.9% of kcal from fat and 0.01% cholesterol) and high-fat/high-cholesterol (HFC) diet group (41.9% of kcal from fat, 1.25% cholesterol). After 8 weeks of ND or HFC diet feeding, the mice were anesthetized with 1%–2% isoflurane in high oxygen and decapitated.

In the second part of the experiment, evaluating the impact of 7β-OHC on AD pathology, we used 39 male C57BL/6 mice sourced from Orient Bio Inc. (Seongnam, Korea). All mice were aged between 16 and 18 weeks at the onset of the experiment and were housed on a 12-h light/dark cycle with food and water available *ad libitum*. For the biological experiment, a total of 17 mice were randomly assigned, with eight mice in the vehicle group and nine in the 7β-OHC group. 7β-OHC was purchased from Sigma (H6891, MW 402.65). The hippocampal administration of 50 μM of 7β-OHC, dissolved in 0.1% ethanol in phosphate-buffered saline (PBS) or vehicle (0.1% ethanol in PBS), was carried out bilaterally using stereotactic techniques, based on specified coordinates relative to the bregma: −2.00 mm anteroposterior (AP), 1.3 mm mediolateral (ML), and 2.2 mm dorsoventral (DV). The injection volume was 0.2 μL for each side of the hippocampus (Fig. S2a). We used the same concentration (50 μM) of free cholesterol (FC), as a putative non-toxic cholesterol to differentiate 7β-OHC toxicity from general toxicity potentially induced by cholesterol molecule itself. Three days post-injection, the mice were euthanized (Fig. S2b). Subsequently, the right cortex and hippocampus were promptly extracted and preserved at −80 °C in a freezer, awaiting Western blotting and ELISA evaluations.

Table 1. Demographics of participants

Variable		NC ($n = 82$)	MCI ($n = 39$)	AD ($n = 81$)	P (ANOVA or χ^2)
Age (years)		71.1 (5.12)	72.7 (5.71)	72.2 (5.23)	0.257
Female, *n* (%)		52 (63.4)	29 (74.4)	59 (72.8)	0.323
Education (years)		7.9 (4.88)	7.7 (4.91)	8.3 (3.87)	0.766
MMSE score		27.6 (1.61)	25.3 (2.52)	21.3 (4.09)	<0.001
GDS score, *n* (%)	1	59 (72)	-	-	<0.001
	2	23 (28)	28 (71.8)	2 (2.5)	
	3	-	11 (28.2)	56 (69.1)	
	4	-	-	14 (17.3)	
	5	-	-	9 (11.1)	
Cholesterol (mg/dL)		190.7 (30.28)	188.7 (44.05)	180.3 (44.36)	0.761
HDLc (mg/dL)		68.0 (31.19)	46.8 (10.40)	49.1 (13.31)	0.065
LDLc (mg/dL)		98.9 (41.02)	115.3 (40.54)	108.4 (38.66)	0.768
Triglyceride (mg/dL)		118.7 (38.89)	115.7 (37.63)	131.5 (71.34)	0.724
Dyslipidemia, *n* (%)		29 (35.8)	15 (38.5)	22 (27.2)	0.360
Statin use, *n* (%)		26 (31.7)	12 (30.8)	21 (25.9)	0.702
Hypertension, *n* (%)		50 (61.0)	25 (64.1)	39 (48.1)	0.145
Diabetes mellitus, *n* (%)		22 (26.8)	5 (12.8)	20 (24.7)	0.219

The data are presented as the mean (SD), *n* (%).

AD, Alzheimer's disease; GDS, global deterioration scale; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; MCI, mild cognitive impairment; MMSE, mini-mental state examination; NC, normal cognition.

Concurrently, the left hemisphere was immediately fixed using 3.7% formaldehyde, preparing it for immunohistochemistry. Furthermore, an additional set of 22 mice was employed for subsequent behavioral experiments and intracerebroventricular infusion was administered to mitigate potential behavioral alterations stemming from tissue damage.

Immunohistochemistry

Frozen hemispheres were fixed and sliced into 20 μm coronal sections with a cryostat. These sections underwent overnight incubation at 4°C with anti-GFAP antibody (1:500; ab173004, Synaptic Systems, Göttingen Germany) and anti-Iba1 antibody (1:100; 019–19,741, Wako, Osaka, Japan) in PBS containing 2% BSA and 0.3% Triton X-100. Subsequently, the slices were treated with Alexa Fluor 488-conjugated donkey anti-goat IgG secondary antibody (1:500;705–545-003, Jackson ImmunoResearch Laboratories, West Grove, PA, USA) for 1 h. The samples were visualized using a confocal laser scanning microscope by Carl Zeiss (Thornwood, NY, USA).

Cytokine production and cell viability assay

The quantification of IL-1β and IL-6 in mouse brain homogenates were determined utilizing mouse IL-1β (ab46052, Abcam, Cambridge, MA, USA) and IL-6 ELISA kits (M6000B, R&D Systems, Minneapolis, MN, USA). Hippocampus homogenates were quantified using the Mouse Cytokine Array Panel A (ARY006; R&D Systems) to profile multiple cytokines and chemokines. All procedures were carried out following the specific protocols of the manufacturers. Nuclear DNA fragmentation associated with apoptosis in tissue sections was evaluated using a double-fluorescent labeling technique, employing a terminal dUTP nick-end labeling (TUNEL) kit (11684795910, Sigma-Aldrich, St. Louis, MO, USA).

Western blotting analyses

The right hemisphere brain tissues were prepared following the methods detailed in reference.[21] Antibodies against the following proteins were used: BACE (1:1:1000; Cell Signaling, #5606S), TACE (1:1000; Santa Cruz; #13,973), APP (1:1000; Cell Signaling, #2450S), Aβ (1:1000; Santa Cruz, #28,365), TNF-α (1:1000; Santa Cruz, #133,192), phospho-tau (Ser262) (1:1000; Santa Cruz, #101,813), phospho-tau (Ser199, Ser202) (1:1000; Invitrogen, #44-768G), phospho-tau 396 (1:1000, Cell Signaling, #9632), phospho-tau 231 (Thr231) (1:1000; Cell Signaling, #71,429), total tau (1:1000; Cell Signaling, #4019), and GAPDH (1:20,000; Santa Cruz, #25,778).

Behavior assessment

Cognitive functions were assessed through a series of behavior tests. In the novel object recognition (NOR) test, 24 h after a 10-min habituation in an open field chamber, the mice initially explored a pair of identical objects for 5 min. After a 1-h interval, the mice were presented with one familiar object and one novel object for 5 min. The Discrimination Index (DI) was calculated using the exploration time for the novel object (T_N) and the familiar object (T_F), employing the formula $DI = (T_N - T_F)/(T_N + T_F)$. The Y maze test was conducted using a Y-shaped maze with three arms arranged at 120°. Mice were allowed to freely explore the maze for 10 min. The alternation percent was calculated by (the number of alternations/[total number of arm entries − 2]) × 100. The behavioral experiment using a touchscreen was conducted with the Bussey-Saksida mouse touchscreen operant system (Campden Instruments Ltd., Loughborough, UK) as noted in previous reports.[22–24] The methods were managed and executed through the ABET II Touch software (Campden Instruments, Ltd.) alongside Whisker Server.[25] We evaluated attention and the ability to control response using the 5-choice serial reaction time task (5-CSRTT). The methodology was implemented as detailed in other reference.[22,26]

Statistical analysis

Statistical evaluations were executed utilizing the Statistical Package for the Social Sciences (SPSS) version 25.0 (SPSS, Inc., Chicago, IL, USA), R Statistical Software version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria), and GraphPad Prism software version 9.50 (San Diego, CA, USA). Group comparisons between 7β-OHC-treated versus vehicle-treated mice ($n < 10$ per group) were conducted using Mann–Whitney *U*-test. However, for experiments with the factorial design (animal steroid profiling and 5-CSRT task), we used linear mixed effects model to explore both main effects and interaction between factors, as demonstrated in our previous studies.[22,27] For studies on humans, the Kruskal–Wallis test assessed differences in hair sterol levels among groups, followed by Dunn's multiple comparison test for *post hoc* analysis. Statistical differences between different groups after adjustment for covariates were analyzed by analysis of covariance on ranks. Owing to data skewness, we conducted multiple linear regression analyses using log-transformed oxysterol values. Covariates considered in the analysis included age, sex, education, and total cholesterol levels. We calculated the odds ratios (ORs) and 95% confidence intervals (CIs) of AD or cognitive impairment for log-transformed 7α/β-OHC levels using binary logistic regression, adjusting for age, sex, education, and total cholesterol levels. Continuous variables are presented as either the means ± standard deviations or medians with interquartile ranges (IQRs). Categorical variables are denoted as quantities with percentages. The threshold for significance was established at 0.05.

Results

Cholesterol metabolite levels significantly changed upon aging in 3xTg-AD mice

Quantitative cholesterol profiling of normal C57BL6 and 3xTg-AD mice of different ages (young *vs*. old) and of mice fed different diets (normal *vs*. HFC) was first conducted. The levels of 17 sterols in the serum, hippocampus, and frontal cortex were measured to identify metabolic changes in cholesterol that show a significant age × genotype interaction (C57BL6 *vs*. 3xTg-AD), preferably with a significant main effect of genotype and diet, since such interactions would indicate AD-specific changes associated with those risk factors (aging or metabolic stress). The metabolic heat-map shows that the serum levels of 7-ketocholesterol, 7-OHC, cholesteryl myristate, cholesteryl arachidonate and the ratio of 7β-OHC/cholesterol were found to meet this criterion (Fig. 1a). In particular, our focus on 7β-OHC, 7-ketocholesterol, and cholesteryl myristate was driven by their connection to oxidative stress and cholesteryl esters have been implicated in AD pathogenesis[28,29] and the near absence of other metabolites in brain tissues. The 7β-OHC/cholesterol ratio in the serum significantly changed in 3xTg-AD mice upon aging, as indicated by the significant main effect of genotype and significant age × genotype interaction (Fig. 1b). However, no corresponding changes were observed in the brain region. Similar changes associated with age and genotype were also observed in serum 7-ketocholesterol/cholesterol levels (Fig. S3a) but not in cholesteryl myristate levels (Fig. S3b). These findings indicate that oxysterols could be peripheral biomarkers of aging-dependent AD pathogenesis.

Cholesterol profiling revealed increased 7-OHC levels in patients with AD

To extend these findings in animals to humans, cholesterol profiling of scalp hair from patients with NC, MCI, and AD was performed. Out of the 19 sterols examined, nine were identified in two 3-cm-long strands of hair (Table 2). The levels of free cholesterol, cholesterol esters, and cholesterol precursors were comparable across groups. Significant between-group differences were found in the levels of sitosterol, 7α-OHC and 7β-OHC ($P = 0.002$, $P = 0.034$, and $P = 0.005$, respectively). As the findings related to oxidized cholesterol levels were consistent between animals and humans, we focused on 7α-OHC and 7β-OHC. First, we compared the levels of these oxysterols according to cognitive status: NC, MCI, and AD.

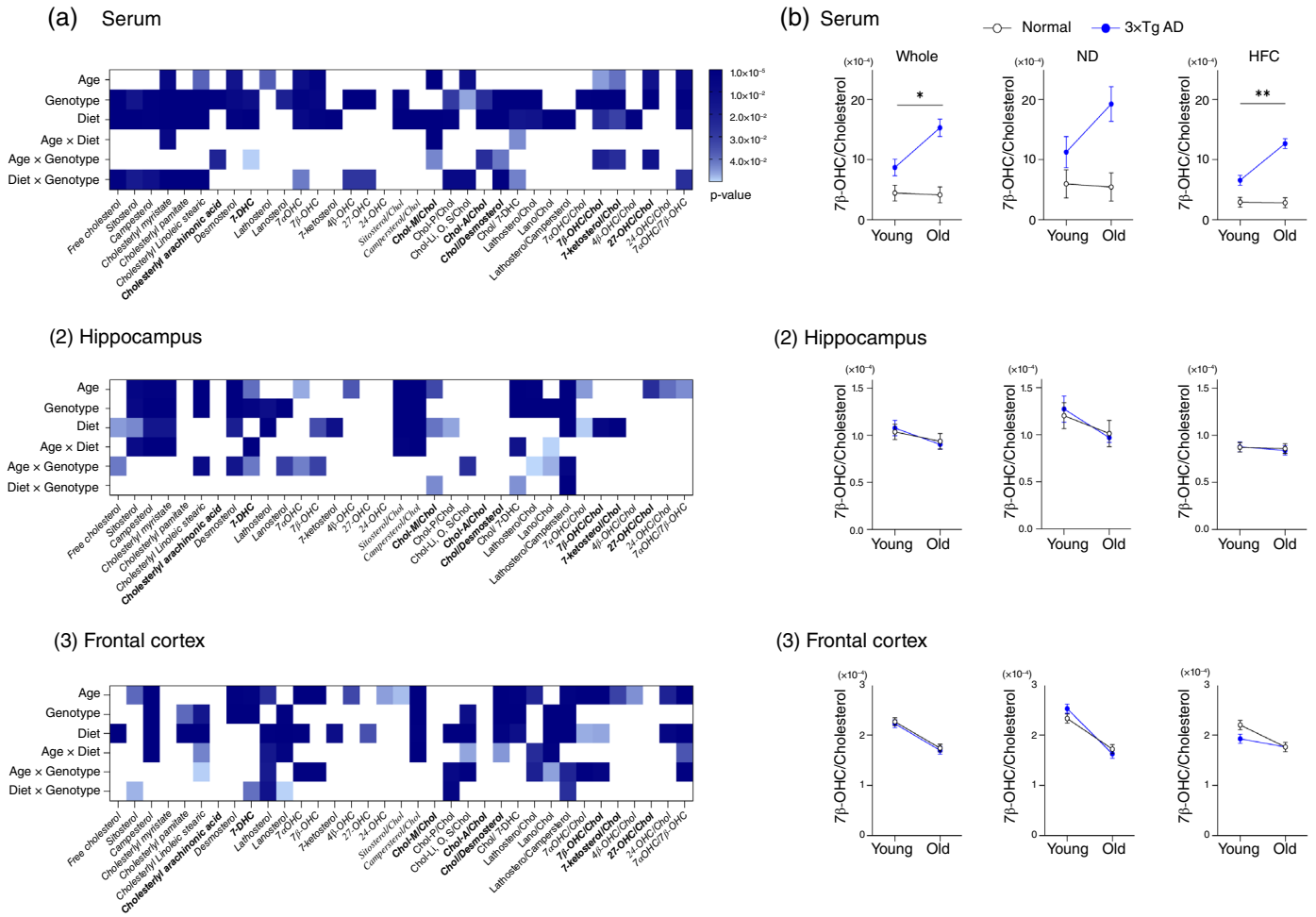

Fig. 1 Steroid profiling of cholesterol metabolites in mice. (a) A heatmap matrix representation of the main effects and interactions between factors (age, genotype, and high-fat/high-cholesterol diet [HFD]). The bolded names indicate a significant age × genotype interaction. (b) Age × genotype interaction on 7β-hydroxycholesterol (OHC)/cholesterol in serum and brain regions. (1) In the whole group ($n = 45$): main effects of age ($P = 0.027$), and genotype ($P < 0.001$), and an age × genotype interaction ($P = 0.016$) in serum. For mice fed a normal diet (ND) ($n = 21$), main effects of genotype ($P = 0.002$) and age ($P = 0.161$) and age × genotype interaction ($P = 0.114$). In mice fed with HFD ($n = 24$), main effects of age ($P = 0.002$) and genotype ($P < 0.001$), and a significant age × genotype interaction ($P = 0.001$) in serum. Main effects of age and genotype, interactions in the (2) hippocampus or (3) frontal cortex (all $P > 0.05$). 7-DHC, 7-dehydrocholesterol; Chol-A, cholesteryl arachidonate; Chol-Li, O, S, cholesteryl linoleate, oleate, stearate; Chol-M, cholesteryl myristate; Chol-P, cholesteryl palmitate; OHC, hydroxycholesterol.

Table 2. The levels of cholesterol derivatives in scalp hair

Compounds (ng)†	NC ($n = 82$)	MCI ($n = 39$)	AD ($n = 81$)	*P* Overall	NC versus MCI	NC versus AD	MCI versus AD
Free cholesterol	609.5 (302.2)	522.4 (303.4)	610.4 (262.5)	0.428	>0.999	>0.999	0.583
Cholesteryl laurate	1.5 (1.9)	1.2 (1.0)	1.5 (1.3)	0.297	0.358	>0.999	0.912
Cholesteryl myristate	21.1 (12.7)	22.0 (13.4)	24.7 (12.4)	0.249	>0.999	0.373	0.660
Sitosterol	1.1 (0.7)	1.2 (0.8)	1.3 (0.9)	0.002*	0.439	0.001*	0.443
Desmosterol	103.5 (48.3)	97.9 (59.6)	98.7 (60.0)	0.469	0.664	>0.999	>0.999
Lathosterol	10.0 (5.9)	7.6 (3.7)	9.5 (5.7)	0.175	0.208	>0.999	0.376
Lanosterol	3.1 (1.1)	3.2 (1.4)	3.4 (1.5)	0.298	>0.999	0.359	>0.999
7α-OHC	1.7 (1.1)	2.1 (1.4)	2.1 (1.3)	0.034*	0.145	0.057	>0.999
7β-OHC	2.5 (1.0)	2.7 (0.8)	2.8 (0.9)	0.005*	0.272	0.004*	>0.999

All values are presented as the median (interquartile range).
AD, Alzheimer's disease; MCI, mild cognitive impairment; NC, normal cognition; OHC, hydroxycholesterol.
*$P < 0.05$.
†The levels measured in two strands of 3-cm long scalp hair. Group differences were determined using the Kruskal–Wallis test followed by Dunn's test for *post hoc* multiple comparison.

The levels of 7α-OHC and 7β-OHC were significantly elevated in patients with AD compared to those with NC. Discernible differences were observed between patients with NC and MCI, although statistical significance was not achieved. Additionally, the levels of 7α-OHC and 7β-OHC relative to total cholesterol were significantly elevated in AD patients compared to NC patients (Fig. 2a). To further substantiate our initial findings, we explored the relationship between oxysterol levels and MMSE and GDS scores, both indicators of clinical severity. Multiple linear regression analyses, adjusted for age, sex, education, and total cholesterol levels, revealed that elevated 7β-OHC levels were correlated with lower MMSE scores ($P = 0.02$) and higher GDS scores ($P < 0.001$), whereas 7α-OHC was significantly associated only with higher GDS scores ($P = 0.03$) (Fig. 2B, Table S1). Further, logistic regression analysis, incorporating both oxysterols, indicated a significant association between 7β-OHC and the risk of AD or cognitive impairment (Fig. 2c). Considering the reported influence of statin use on oxysterol levels,[30,31] we additionally included statin use as a covariate and found that significance of the results remained consistent. To assess the diagnostic utility of hair 7β-OHC levels, we generated ROC curves and developed a model for discriminating between diagnostic groups. Of note, the combined panel of MMSE and hair 7β-OHC levels resulted in a more pronounced AUC of 0.98 ($P = 0.03$, Fig. 2d) for differentiating AD from NC, compared to the MMSE score by itself with an AUC of 0.96. This finding suggests that implementation of a hair 7β-OHC assay in addition to the MMSE can facilitate more efficient detection of AD patients in community settings.

7β-OHC induces neuroinflammation

As we observed a significant relationship between 7β-OHC levels and AD, we aimed to determine whether 7β-OHC can directly affect AD pathology. Upon stereotactic injection of 7β-OHC, compared to vehicle and FC, into the mouse hippocampus, we observed glial cell activation, as indicated by morphological changes in glial cells (Fig. 3a). An increase in GFAP and Iba-1 intensity was observed in the CA1, CA3 and DG regions upon 7β-OHC treatment. Cytokine array revealed increases in the levels of TNF-α, TIMP-1 and I-TAC in 7β-OHC treated mice, compared with vehicle treated mice (Figs 3b, S4a,b). The ability of 7β-OHC to induce neuroinflammation was supported by increases in the levels of pro-inflammatory cytokines such as IL-1β and IL-6 (Fig. 3c). To rule out the possibility that 7β-OHC-induced cell death triggered neuroinflammation, we further evaluated apoptosis using TUNEL staining. No significant cell death was observed in hippocampal tissues from 7β-OHC-treated mice compared with those from vehicle-treated mice, suggesting that 7β-OHC is directly involved in neuroinflammation (Fig. S5).

7β-OHC increases Aβ production and tau phosphorylation

We also examined the effect of 7β-OHC on Aβ metabolism by measuring the expression levels of proteins involved in the amyloidogenic pathway.[32,33] 7β-OHC significantly increased BACE1 expression but decreased TACE expression (Fig. 3d). No significant changes were observed in the expression levels of Aβ, APP, SIRT1, or ADAM10. These findings indicate that 7β-OHC stimulates the amyloidogenic pathway (BACE1) while suppressing the nonamyloidogenic pathway. Next, we examined tau phosphorylation at multiple sites, i.e., Ser262 (p-Tau262), Ser396 (p-Tau396), Ser199/202 (p-Tau199/202), and Thr231 (p-Tau231), all of which have been implicated in the pathogenesis of AD.[34] With 7β-OHC treatment, we observed an elevation in p-Tau262 levels, but there was no corresponding increase in the phosphorylation of tau at other sites or in total Tau levels (Fig. S6), indicating that 7β-OHC might selectively induce the phosphorylation of Tau at Ser262, which inhibits tau binding to microtubules.[35]

Elevated levels of 7β-OHC resulted in impairments in memory and executive functions

Finally, to evaluate the influence of 7β-OHC on cognitive and behavioral outcomes, we administered the novel object recognition (NOR) task, Y-maze task, and the 5-CSRTT after the injection of 7β-OHC. The novel object test revealed that episodic memory was impaired in 7β-OHC-injected group compared to control group (Fig. 3e). No significant between-group difference was found in Y-maze test (Fig. 3f). Considering that attention and response control are compromised in

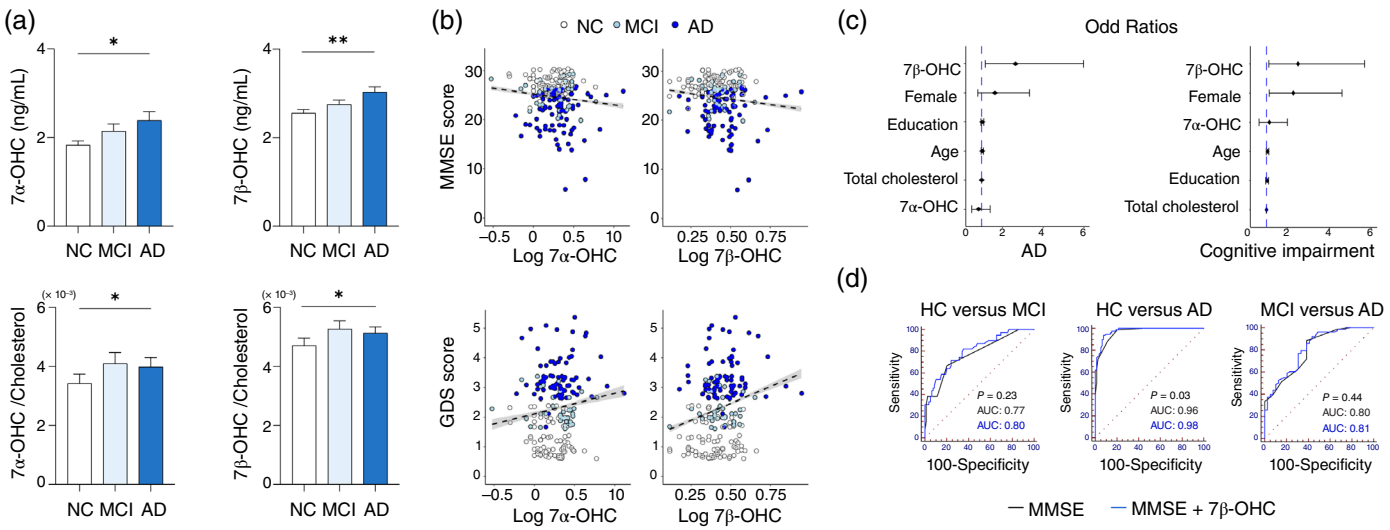

Fig. 2 Hair 7-hydroxycholesterols and cognitive status. (a) Pairwise comparisons between groups show significantly higher levels of hair 7α-OHC and 7β-OHC in AD participants than in NC participants ($P = 0.020$ and $P = 0.003$, respectively, Dunnett adjustment for multiple comparisons). The levels of 7α-OHC/cholesterol and 7β-OHC/cholesterol were increased according to the clinical diagnosis ($P = 0.014$, $P = 0.025$, respectively). (b) Dashed line indicates predicted MMSE and GDS from log transformed 7α-OHC and 7β-OHC by multiple linear regression under the control of age, sex, education, and total cholesterol levels. (c) Forest plot for binary logistic regression analysis of 7-oxysterol levels and AD risk. 7β-OHC levels are associated with risk of both AD and cognitive impairment (NC *vs*. MCI, AD). (d) ROC curves of the combination of hair 7β-OHC levels and MMSE scores for AD patients. The solid lines of different colors (black for MMSE scores and blue for MMSE scores +7β-OHC levels) show the estimated ROC curves for the univariate prediction rule. *$P < 0.05$; **$P < 0.01$ indicate significance. AD, Alzheimer's disease; GDS, Global deterioration scale; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NC, normal cognition; OHC, hydroxycholesterol; ROC, Receiver operating characteristic.

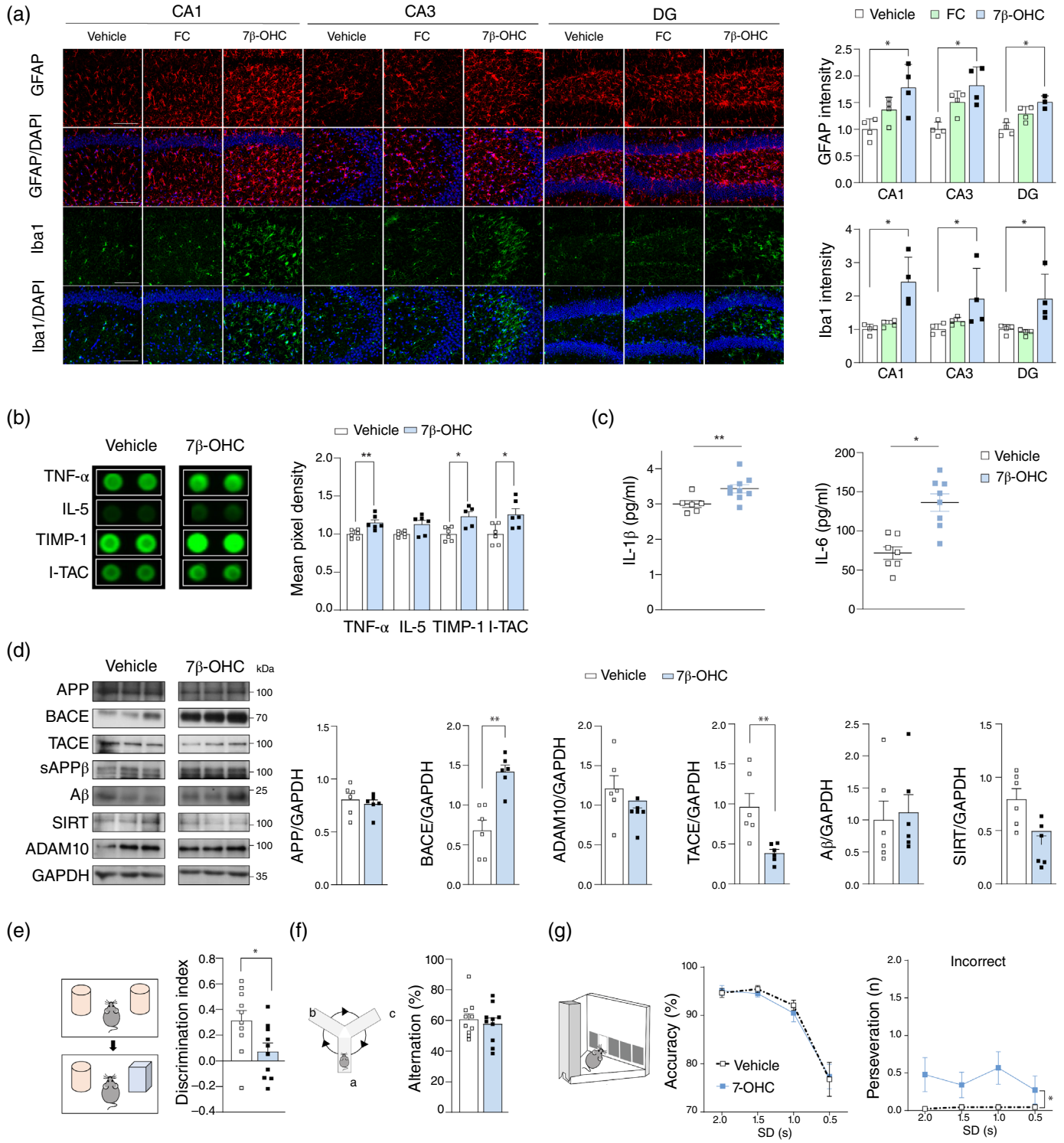

Fig. 3 7β-OHC induces neuroinflammation, the amyloidogenic pathway, and cognitive dysfunction. (a) Immunostaining of GFAP (red), Iba-1 (green) and DAPI (blue) in the hippocampal regions ($n = 4$). Scale bar, 20 μm, and quantification of its intensity. (b) Cytokines in the hippocampus ($n = 4$ per group) changed significantly when measured by Multiple Mouse Cytokine Array. (c) The proinflammatory cytokines IL-1β and IL-6 measured using ELISA. (d) Representative western blot and quantification of enzymes involved in the amyloidogenic pathway following intrahippocampal injection of 7β-OHC ($n = 6$ per group). (e,f) Novel Object Recognition and Y-maze tests conducted on 3 days post intrahippocampal 7β-OHC injection ($n = 10$). (g) 5-choice serial reaction time task upon 7β-OHC intracerebroventricular injection ($n = 11$ per group). The data are presented as the mean ± S.E.M. $^{*}P < 0.05$; $^{**}P < 0.01$. 7β-OHC, 7β-hydroxycholesterol; ADAM 10, A disintegrin and metalloproteinase domain-containing protein 10; APP, amyloid precursor protein; BACE, beta-site APP cleaving enzyme; CA, cornu amonis; DG, dentate gyrus; FC, free cholesterol; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TACE, tumor necrosis factor-α–converting enzyme.

the early stages of AD,[36] we also investigated their effects on attention and executive function using 5-CSRTT.[37] In baseline sessions after stereotaxic surgery, all mice met the criterion to move on to the probe test, indicating no between-group differences at baseline (Fig. S7a–e). In the probe sessions with more difficult task conditions (shorter SDs), accuracy and omission did not differ between groups

(Figs 3g, S8a–e). However, the injection of 7β-OHC resulted in cognitive inflexibility, as evidenced by the elevated number of perseverative responses to incorrect stimuli, but not to correct stimuli (Figs 3g, S8a). The observed behavioral deficit cannot be attributed to impairments in locomotion, as beam break rates and reward collection latencies remained unaffected following the injection of 7β-OHC (Fig S8f,g). Thus, these findings indicate that 7β-OHC induces frontal/executive dysfunction,[38,39] which might cause irrelevant, repetitive behaviors, specifically when mice are facing an unexpected lack of a reward upon responding.

Discussion

This study aimed to identify which cholesterol metabolite is associated with AD and to elucidate its potential role in AD pathogenesis. We discovered that the levels of 7β-OHC were elevated in the serum of old, but not young, 3xTg-AD mice and in the scalp hair of patients with AD. In humans, the levels of 7β-OHC were negatively correlated with MMSE scores, and the stereotaxic injection of 7β-OHC *in vivo* led to marked neuroinflammation and upregulation of amyloidogenic pathway. These pronounced alterations were directly associated with observed deficits in memory and executive functions, as corroborated by behavior tests. These findings suggest that 7β-OHC could be a pathophysiologically relevant peripheral biomarker of AD.

Our results are consistent with recent findings that 7β-OHC levels are increased in the brain tissues of patients with AD, supporting the role of this molecule as a promising biomarker of AD.[14,40] We observed the impact on behaviors (memory) of the injected levels of 7β-OHC equivalent to twice the physiological concentration found in normal mice and also significant age by genotype interaction on the serum levels of this molecule. However, we did not observe significant genotype effects on 7β-OHC levels in the brain tissues (normal *vs*. 3xTg-AD mice). We speculate that the age of 3xTg-AD mice (11–12 months) might account for this discrepancy: Cholesterol metabolism might not yet be substantially perturbed in the brain, unlike in the systemic circulation. Or this inconsistency between serum and the brain tissue may imply that cholesterol homeostasis in the brain may be under stricter control than in the periphery, given the brain's metabolic vulnerability.[41]

On the other hand, this result might be in line with the hypothesis of "blood-driven dementia," which suggests the importance of blood factors in AD pathogenesis.[42] This hypothesis proposes that peripheral pathology may precede central pathology. Several experimental and epidemiological studies have suggested that cholesterol dysregulation in middle age can lead to AD development later in life.[5,43,44] A recent study further demonstrated a significant correlation between brain/CSF 7β-OHC levels (with 7-ketocholesterol) and Braak staging.[40] Unlike free cholesterol, oxysterol can traverse the BBB and accumulate in brain tissue,[45,46] and elevated oxysterols levels may in turn impact the integrity of BBB.[47,48] Hence, it remains unclear whether previously reported accumulation of 7β-OHC in the brain originates from the periphery or through the auto-oxidation of cholesterol within the brain (possibly driven by Aβ[49]). Given the tight regulation of cholesterol homeostasis, one may speculate that 7β-OHC is tightly regulated in brain to a certain extent; however, when overall 7β-OHC levels becomes elevated systemically, it could potentially trigger chronic inflammation and oxidative stress in the brain, thereby heightening the risk of dementia.

While 7β-OHC promoted the amyloidogenic pathway as indicated by the upregulated BACE1 expression, a significant increase in Aβ levels was not simultaneously observed. As Aβ accumulation is not solely dependent on BACE1 activity but also on age-associated imbalance between its production and clearance,[50,51] the relatively young age of our mice may have limited meaningful changes in Aβ levels under our experimental scheme. However, it should be noted that we did not examine different species of Aβ, from monomers to oligomers or soluble to insoluble forms. Regarding the behavioral outcomes, we observed impaired performance in the NOR but not in the Y-Maze test. This disparity might be attributed to the potential difference in region of the brain mainly involved in each task and our local (not systemic injection of 7β-OHC). As the perirhinal cortex and hippocampus are involved in episodic or object memory in the NOR test, the impact of hippocampal injection of 7β-OHC would have been more evident in this task compared to the Y-maze in which working memory could have relied on the prefrontal function.[52,53]

In this study, hair levels of 7β-OHC were measured instead of human blood levels, which may fluctuate due to diurnal variations or diet, and the levels of sterols in hair samples reflect sterol exposure over approximately the past 3 months.[54] To minimize local variation of hair steroid levels along with different parts of the head,[55] hair samples were obtained from the vertex of each participant in this study. Therefore, the hair 7β-OHC level could serve as a reliable indicator of systemic exposure. In addition, as hair sampling is extremely convenient and safe, analysis of hair 7β-OHC levels is a promising candidate strategy for implementation in mass screening in community settings. Although natural color and pigmentation of hair showed no differences for hair levels of steroids,[56,57] excessive cosmetic treatments and prolonged exposure to UV light may need to be considered as confounding effects.[57,58] Implementation of this convenient biomarker combined with the MMSE conforms to the recently proposed AD diagnostic approach: diagnostic accuracy is being sought on the basis of "cognitive assessment plus biomarker." We found that the combination of the MMSE + hair 7β-OHC level analysis achieved a higher AUC than the MMSE alone. A large population study is warranted to validate the usefulness of the combination of the MMSE + hair 7β-OHC level analysis for dementia screening in the community.

The finding that 7β-OHC upregulates BACE1 expression suggests the possibility of a vicious cycle between 7β-OHC and Aβ pathology, given that a previous study showed that APP and Aβ can oxidize free cholesterol into 7β-OHC.[49] In our experiment, significant increase in tau phosphorylation was observed only at Ser262 among various sites. Mitogen-activated protein kinase (MAPK) or other proline-directed kinases are involved in tau phosphorylation; however, Ser262 is not phosphorylated by these kinases.[59] Thus, the selective increase in pTau262 suggests that 7β-OHC-induced phosphorylation of tau might be less likely to be mediated by the common tau pathogenesis relating to MAPK pathway. It remains to be determined whether 7β-OHC directly affects AD pathology by causing oxidative stress in the brain or merely reflects the level of systemic oxidative stress in AD, as cholesterol is highly sensitive to auto-oxidation by free radicals. However, as we proved that 7β-OHC contributes to the hallmarks of AD pathology, controlling systemic 7β-OHC levels could be a promising strategy for preventing and ameliorating AD pathogenesis, at least by blocking the potential vicious cycle.

Statins could be a potential option for this purpose, and simvastatin has been shown to decrease the levels of 7β-OHC in men with hypercholesterolemia.[30] Previous studies exploring the effectiveness of statin use on the suppression of AD have reported inconsistent findings.[60–62] However, these conflicting results may suggest that measurement of 7β-OHC levels is a potential avenue for personalized medicine for AD, as levels of 7β-OHC can be used to identify those who are eligible for statin therapy to inhibit AD pathology. As not all individuals with hypercholesterolemia suffer from AD, 7β-OHC might be the real culprit responsible for the increased risk of AD in individuals with hypercholesterolemia.

We need to acknowledge several limitations in this study. Firstly, the sample size of human participants was relatively small and obtained from a single ethnic background. Additionally, while we explored the relationship between cognitive measures like MMSE and GDS scores and their link to 7β-OHC, we did not establish correlations with direct indicators of amyloid pathology, such as cerebrospinal fluid amyloid beta protein levels or amyloid positron emission tomography (PET) scans. Therefore, further research is warranted to confirm and extend our findings to a more diverse population with more clinical information related to AD pathology. Secondly, the effect of 7β-OHC on oxidative stress was not thoroughly investigated in this study. Previous research has suggested that 7β-OHC can trigger oxidative stress in murine oligodendrocytes.[63] Considering that oxidative stress is one of

the major etiologies of AD pathogenesis, it is plausible that oxidative stress induced by 7β-OHC could contribute to the promotion of the amyloidogenic pathway. Additional investigations are required to comprehensively address this aspect. Another concern is whether the concentration of 7β-OHC we injected is associated with actual pathological conditions. Postmortem studies have indicated that 7β-OHC levels in the brains of AD patients are more than double compared to normal conditions, suggesting its close association with AD pathology.[14,40] In our experiment, we administered nearly double the physiological concentration of 7β-OHC and observed its impact on AD pathology and behavior under acute conditions. Additionally, we confirmed that this effect is not attributed to cell death and emphasized its significance compared to free cholesterol administration. Based on these, the concentration of 7β-OHC we used may not be too high to study its relevance with AD pathogenesis. Although we examined the acute effects of 7β-OHC, it would be conceivable that lower concentrations could induce AD pathology with prolonged exposure. Lastly, we did not conduct steroid profiling of the serum or assess apolipoprotein E status in the participants. As the purpose of this study was to swiftly extend the animal findings to humans, we intended to perform a simplified human study. Thus, future clinical studies should be designed to obtain more extensive information by neuroimaging and blood biomarker analysis.

In summary, this study shows that 7β-OHC could be a promising biomarker of AD that directly affects AD pathogenesis, such as neuroinflammation, Aβ metabolism, and tau phosphorylation. Therefore, the implication of this study is twofold for the future. First, implementation of a 7β-OHC assay for AD screening in the community may improve the efficiency in identifying individuals at high risk. Second, using 7β-OHC as a biomarker in the clinical realm may facilitate personalized medicine in which younger individuals with high levels of 7β-OHC can be selected to receive statin or antioxidant therapy to prevent or ameliorate AD pathology.

Acknowledgments

The authors thank all the participants who voluntarily agreed to take part in this study. The authors also thank Mr. Dong-Su Jang and the Medical Illustration & Design, a part of the Medical Research Support Services of Yonsei University College of Medicine, for all artistic support related to this work. A part of the results from this study was presented at the Alzheimer's Association International Conference, 2020.

Disclosure statement

E.K. is paid as a member of the scientific advisory board for Amyloid Solution, Inc. and AevisBio, Inc.

Author contributions

J.H. and E.K. designed the study and conceptualized it, J.H. analyzed and interpreted the clinical data, led the molecular study, and drafted the manuscript. G.E.K. performed cholesterol profiling and Y.S. and S.A.J. played a major role in acquisition of the data. S.Y.C., J.M.L. performed animal experiment and interpreted data, and J.L., D.S. assisted in the behavior experiments. H.K., S.J.P had role in conceptualization and interpreted the data. M.L. and D.Y.L. provided advice on statistical analysis for intellectual content. M.H.C. and E.K. supervised in conceptualization and acquisition, and reviewed manuscript. All authors read and approved the final manuscript.

Funding information

This study was supported by grants from Research Driven Hospitals Development R&D Project (HR22C141102) funded by the Ministry of Health and Welfare, Korea, and the Source Technology Development Project (2022M3E5E8030792) funded by National Research Foundation (NRF) of Korea, the Ministry of Science and ICT, and Korea Environment Industry & Technology Institute (KEITI) through Core Technology Development Project (No.2022003310011) for Environmental Diseases Prevention and Management, funded by Korea Ministry of Environment, and Basic Research Laboratory (BRL) project (RS-2023-00207834) funded by NRF of Korea.

References

1. Puglielli L, Tanzi RE, Kovacs DM. Alzheimer's disease: The cholesterol connection. *Nat. Neurosci.* 2003; **6**: 345–351.
2. Casserly I, Topol E. Convergence of atherosclerosis and Alzheimer's disease: Inflammation, cholesterol, and misfolded proteins. *Lancet* 2004; **363**: 1139–1146.
3. Puglielli L, Konopka G, Pack-Chung E *et al*. Acyl-coenzyme a: Cholesterol acyltransferase modulates the generation of the amyloid beta-peptide. *Nat. Cell Biol.* 2001; **3**: 905–912.
4. Granholm A-C, Bimonte-Nelson HA, Moore AB, Nelson ME, Freeman LR, Sambamurti K. Effects of a saturated fat and high cholesterol diet on memory and hippocampal morphology in the middle-aged rat. *J. Alzheimers Dis.* 2008; **14**: 133–145.
5. Kivipelto M, Helkala EL, Laakso MP *et al*. Midlife vascular risk factors and Alzheimer's disease in later life: Longitudinal, population based study. *BMJ* 2001; **322**: 1447–1451.
6. Iwagami M, Qizilbash N, Gregson J *et al*. Blood cholesterol and risk of dementia in more than 1.8 million people over two decades: A retrospective cohort study. *Lancet Healthy Longev.* 2021; **2**: e498–e506.
7. Tan ZS, Seshadri S, Beiser A *et al*. Plasma total cholesterol level as a risk factor for Alzheimer disease: The Framingham study. *Arch. Intern. Med.* 2003; **163**: 1053–1057.
8. Mielke MM, Zandi PP, Shao H *et al*. The 32-year relationship between cholesterol and dementia from midlife to late life. *Neurology* 2010; **75**: 1888–1895.
9. Proitsi P, Kim M, Whiley L *et al*. Plasma lipidomics analysis finds long chain cholesteryl esters to be associated with Alzheimer's disease. *Transl. Psychiatry* 2015; **5**: e494.
10. van der Kant R, Langness VF, Herrera CM *et al*. Cholesterol metabolism is a druggable Axis that independently regulates tau and amyloid-β in iPSC-derived Alzheimer's disease neurons. *Cell Stem Cell* 2019; **24**: 363–375.e369.
11. Goedeke L, Fernández-Hernando C. Regulation of cholesterol homeostasis. *Cell. Mol. Life Sci.* 2012; **69**: 915–930.
12. Maxfield FR, Tabas I. Role of cholesterol and lipid organization in disease. *Nature* 2005; **438**: 612–621.
13. Hascalovici JR, Vaya J, Khatib S *et al*. Brain sterol dysregulation in sporadic AD and MCI: Relationship to heme oxygenase-1. *J. Neurochem.* 2009; **110**: 1241–1253.
14. Testa G, Staurenghi E, Zerbinati C *et al*. Changes in brain oxysterols at different stages of Alzheimer's disease: Their involvement in neuroinflammation. *Redox Biol.* 2016; **10**: 24–33.
15. Varma VR, Büşra Lüleci H, Oommen AM *et al*. Abnormal brain cholesterol homeostasis in Alzheimer's disease—A targeted metabolomic and transcriptomic study. *NPJ Aging Mech Dis.* 2021; **7**: 11.
16. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed., text rev. edn. American Psychiatric Association, Washington, DC, 2000.
17. Petersen RC. Mild cognitive impairment as a diagnostic entity. *J. Intern. Med.* 2004; **256**: 183–194.
18. Son HH, Lee DY, Seo HS *et al*. Hair sterol signatures coupled to multivariate data analysis reveal an increased 7beta-hydroxycholesterol production in cognitive impairment. *J. Steroid Biochem. Mol. Biol.* 2016; **155**: 9–17.
19. Cho AR, Moon JY, Kim S *et al*. Effects of alternate day fasting and exercise on cholesterol metabolism in overweight or obese adults: A pilot randomized controlled trial. *Metabolism* 2019; **93**: 52–60.
20. Cho HL, Kim JH, Ryu SM, Noh J, Lee SW, Choi MH. Interactive metabolic signatures of testicular testosterone with bilateral adrenalectomy in mice. *J. Steroid Biochem. Mol. Biol.* 2023; **231**: 106333.
21. Park SJ, Lee J, Lee S *et al*. Exposure of ultrafine particulate matter causes glutathione redox imbalance in the hippocampus: A neurometabolic susceptibility to Alzheimer's pathology. *Sci. Total Environ.* 2020; **718**: 137267.
22. White MA, Kim E, Duffy A *et al*. TDP-43 gains function due to perturbed autoregulation in a Tardbp knock-in mouse model of ALS-FTD. *Nat. Neurosci.* 2018; **21**: 552–563.
23. Mar AC, Horner AE, Nilsson SRO *et al*. The touchscreen operant platform for assessing executive function in rats and mice. *Nat. Protoc.* 2013; **8**: 1985–2005.

24. Kim EW, Phillips BU, Heath CJ *et al*. Optimizing reproducibility of operant testing through reinforcer standardization: Identification of key nutritional constituents determining reward strength in touchscreens. *Mol. Brain* 2017; **10**: 31.
25. Cardinal RN, Aitken MR. Whisker: A client—Server high-performance multimedia research control system. *Behav. Res. Methods* 2010; **42**: 1059–1071.
26. Lee JH, Cho SY, Kim E. Translational cognitive neuroscience of dementia with touchscreen operant chambers. *Genes Brain Behav.* 2021; **20**: e12664.
27. Cho SY, Kim EW, Park SJ *et al*. Reconsidering repurposing: Long-term metformin treatment impairs cognition in Alzheimer's model mice. *Transl. Psychiatry* 2024; **14**: 34.
28. Di Paolo G, Kim T-W. Linking lipids to Alzheimer's disease: Cholesterol and beyond. *Nat. Rev. Neurosci.* 2011; **12**: 284–296.
29. Iriondo A, García-Sebastian M, Arrospide A *et al*. Cerebrospinal fluid 7-Ketocholesterol level is associated with amyloid-β42 and White matter microstructure in cognitively healthy adults. *J. Alzheimers Dis.* 2020; **76**: 643–656.
30. Dias IHK, Milic I, Lip GYH, Devitt A, Polidori MC, Griffiths HR. Simvastatin reduces circulating oxysterol levels in men with hypercholesterolaemia. *Redox Biol.* 2018; **16**: 139–145.
31. Passarelli MN, McDonald JG, Thompson BM *et al*. Association of demographic and health characteristics with circulating oxysterol concentrations. *J. Clin. Lipidol.* 2022; **16**: 345–355.
32. Querfurth HW, LaFerla FM. Alzheimer's disease. *N. Engl. J. Med.* 2010; **362**: 329–344.
33. Wang R, Li JJ, Diao S *et al*. Metabolic stress modulates Alzheimer's β-secretase gene transcription via SIRT1-PPARγ-PGC-1 in neurons. *Cell Metab.* 2013; **17**: 685–694.
34. Johnson GV, Stoothoff WH. Tau phosphorylation in neuronal cell function and dysfunction. *J. Cell Sci.* 2004; **117**: 5721–5729.
35. Sengupta A, Kabat J, Novak M, Wu Q, Grundke-Iqbal I, Iqbal K. Phosphorylation of tau at both Thr 231 and ser 262 is required for maximal inhibition of its binding to microtubules. *Arch. Biochem. Biophys.* 1998; **357**: 299–309.
36. Perry RJ, Hodges JR. Attention and executive deficits in Alzheimer's disease: A critical review. *Brain* 1999; **122**: 383–404.
37. Romberg C, Bussey TJ, Saksida LM. Paying more attention to attention: Towards more comprehensive cognitive translation using mouse models of Alzheimer's disease. *Brain Res. Bull.* 2013; **92**: 49–55.
38. Lim J, Kim E, Noh HJ *et al*. Assessment of mGluR5 KO mice under conditions of low stress using a rodent touchscreen apparatus reveals impaired behavioural flexibility driven by perseverative responses. *Mol. Brain* 2019; **12**: 37.
39. Kim E, White MA, Phillips BU *et al*. Coexistence of perseveration and apathy in the TDP-43Q331K knock-in mouse model of ALS–FTD. *Transl. Psychiatry* 2020; **10**: 377.
40. Dias IHK, Shokr H, Shephard F, Chakrabarti L. Oxysterols and oxysterol sulfates in Alzheimer's disease brain and cerebrospinal fluid. *J. Alzheimers Dis.* 2022; **87**: 1527–1536.
41. Milstein JL, Ferris HA. The brain as an insulin-sensitive metabolic organ. *Mol Metab.* 2021; **52**: 101234.
42. Rieux M, Alpaugh M, Cicchetti F. The troubling story of blood-driven dementias. *Mol. Psychiatry* 2019; **24**: 162–166.
43. Solomon A, Kivipelto M, Wolozin B, Zhou J, Whitmer RA. Midlife serum cholesterol and increased risk of Alzheimer's and vascular dementia three decades later. *Dement. Geriatr. Cogn. Disord.* 2009; **28**: 75–80.
44. Refolo LM, Pappolla MA, Malester B *et al*. Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol. Dis.* 2000; **7**: 321–331.
45. Heverin M, Meaney S, Lütjohann D, Diczfalusy U, Wahren J, Björkhem I. Crossing the barrier: Net flux of 27-hydroxycholesterol into the human brain. *J. Lipid Res.* 2005; **46**: 1047–1052.
46. Leoni V, Masterman T, Mousavi FS *et al*. Diagnostic use of cerebral and extracerebral oxysterols. *Clin. Chem. Lab. Med.* 2004; **42**: 186–191.
47. Dias IH, Polidori MC, Griffiths HR. Hypercholesterolaemia-induced oxidative stress at the blood-brain barrier. *Biochem. Soc. Trans.* 2014; **42**: 1001–1005.
48. Leoni V, Masterman T, Patel P, Meaney S, Diczfalusy U, Björkhem I. Side chain oxidized oxysterols in cerebrospinal fluid and the integrity of blood-brain and blood-cerebrospinal fluid barriers. *J. Lipid Res.* 2003; **44**: 793–799.
49. Nelson TJ, Alkon DL. Oxidation of cholesterol by amyloid precursor protein and beta-amyloid peptide. *J. Biol. Chem.* 2005; **280**: 7377–7387.
50. Mawuenyega KG, Sigurdson W, Ovod V *et al*. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science* 2010; **330**: 1774.
51. Patterson BW, Elbert DL, Mawuenyega KG *et al*. Age and amyloid effects on human central nervous system amyloid-beta kinetics. *Ann. Neurol.* 2015; **78**: 439–453.
52. Cohen SJ, Stackman RW Jr. Assessing rodent hippocampal involvement in the novel object recognition task. A review. *Behav. Brain Res.* 2015; **285**: 105–117.
53. Kraeuter A-K, Guest PC, Sarnyai Z. The Y-maze for assessment of spatial working and reference memory in mice. **In:** Guest PC (ed.). *Pre-Clinical Models: Techniques and Protocols*. Springer, New York, NY, 2019; 105–111.
54. Lee DY, Kim E, Choi MH. Technical and clinical aspects of cortisol as a biochemical marker of chronic stress. *BMB Rep.* 2015; **48**: 209–216.
55. Hobo Y, Nishikawa J, Miyashiro Y, Fujikata A. Analysis of hair steroid hormone concentrations at different parts of the head by liquid chromatography-tandem mass spectrometry. *Clin. Chim. Acta* 2021; **523**: 260–266.
56. Voegel CD, Hofmann M, Kraemer T, Baumgartner MR, Binz TM. Endogenous steroid hormones in hair: Investigations on different hair types, pigmentation effects and correlation to nails. *Steroids* 2020; **154**: 108547.
57. Preinbergs JK, Sundström-Poromaa I, Theodorsson E, Ström JO, Ingberg E. Effect of cosmetic hair treatment and natural hair colour on hair testosterone concentrations. *PLoS One* 2023; **18**: e0291812.
58. Hodes A, Lodish MB, Tirosh A *et al*. Hair cortisol in the evaluation of Cushing syndrome. *Endocrine* 2017; **56**: 164–174.
59. Biernat J, Gustke N, Drewes G, Mandelkow E, Mandelkow E. Phosphorylation of Ser262 strongly reduces binding of tau to microtubules: Distinction between PHF-like immunoreactivity and microtubule binding. *Neuron* 1993; **11**: 153–163.
60. Jick H, Zornberg GL, Jick SS, Seshadri S, Drachman DA. Statins and the risk of dementia. *Lancet* 2000; **356**: 1627–1631.
61. Schultz BG, Patten DK, Berlau DJ. The role of statins in both cognitive impairment and protection against dementia: A tale of two mechanisms. *Transl. Neurodegener.* 2018; **7**: 5.
62. Olmastroni E, Molari G, De Beni N *et al*. Statin use and risk of dementia or Alzheimer's disease: A systematic review and meta-analysis of observational studies. *Eur. J. Prev. Cardiol.* 2021; **29**: 804–814.
63. Sghaier R, Nury T, Leoni V *et al*. Dimethyl fumarate and monomethyl fumarate attenuate oxidative stress and mitochondrial alterations leading to oxiapoptophagy in 158N murine oligodendrocytes treated with 7β-hydroxycholesterol. *J. Steroid Biochem. Mol. Biol.* 2019; **194**: 105432.

Supporting Information

Additional supporting information can be found online in the Supporting Information section at the end of this article.