27-hydroxycholesterol promotes oligodendrocyte maturation: Implications for hypercholesterolemia-associated brain white matter changes

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Abstract
Oxidized cholesterol metabolite 27-hydroxycholesterol (27-OH) is a potential link between hypercholesterolemia and neurodegenerative diseases since unlike peripheral cholesterol, 27-OH is transported across the blood–brain barrier. However, the effects of high 27-OH levels on oligodendrocyte function remain unexplored. We hypothesize that during hypercholesterolemia 27-OH may impact oligodendrocytes and myelin and thus contribute to the disconnection of neural networks in neurodegenerative diseases. To test this idea, we first investigated the effects of 27-OH in cultured oligodendrocytes and found that it induces cell death of immature O4+ /GalC+ oligodendrocytes along with stimulating differentiation of PDGFRα+ oligodendrocyte progenitors (OPCs). Next, transgenic mice with increased systemic 27-OH levels (Cyp27Tg) underwent behavioral testing and their brains were immunohistochemically stained and lysed for immunoblotting. Chronic exposure to 27-OH in mice

Abbreviations:
27-OH, 27-hydroxycholesterol; Aβ, amyloid-beta; AD, Alzheimer’s disease; BBB, blood–brain barrier; CC1, adenomatous polyposis coli clone 1; CNPase, 2'-3'-cyclic-nucleotide 3'- phosphodiesterase; CSF, cerebrospinal fluid; CYP27A1, human sterol 27-hydroxylase; Cyp27Tg, human sterol 27-hydroxylase overexpressing mice; DIV, days-in-vitro; FC, fear conditioning; IF, immunofluorescence; LFB, Luxol fast blue; MBP, myelin basic protein; MMSE, mini mental state examination; MOG, myelin oligodendrocyte glycoprotein; MWM, Morris water maze; NGS, normal goat serum; Olig2, oligodendrocyte transcription factor 2; OPC, oligodendrocyte progenitor cell; PDGFRα, platelet-derived growth factor receptor α; PLP, myelin proteolipid protein; p-Tau, phosphorylated tau; RAVLT, Rey Auditory Verbal Learning Test; Tg-, non-transgenic littermates of Cyp27Tg mice; t-Tau, total tau.

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INTRODUCTION

Factors related to lifestyle play a pivotal role in increasing the risk of developing neurodegenerative disorders (Lisko et al., 2020). Midlife hypercholesterolemia has been linked to both late- and early-onset Alzheimer’s disease (AD) (Anstey et al., 2017; Wingo et al., 2019), and hypercholesterolemia is also one of the risk factors for vascular dementia (Appleton et al., 2017). The role of cholesterol metabolism in neurodegeneration is further corroborated by the association between familial hypercholesterolemia and cognitive deficits (Zambon et al., 2010). Nonetheless, cholesterol is unable to penetrate the blood–brain barrier (BBB) and therefore the brain synthesizes cholesterol de novo (Jurevics & Morell, 1995). Yet there are secondary vascular factors linking high systemic cholesterol to neurodegenerative changes, such as atherosclerosis and lesions of the white matter (de Bem et al., 2021; Tong et al., 2019). A more direct link between high systemic cholesterol and brain changes may be oxysterols, which are metabolites of cholesterol emerging in research as biologically active compounds. Unlike cholesterol, oxysterols can cross the BBB (Björkhem et al., 1998; Heverin et al., 2005).

The peripherally abundant 27-hydroxycholesterol (27-OH) has been associated with AD (reviewed in Björkhem et al., 2006; Loera-Valencia et al., 2019). 27-OH is produced by the enzyme sterol 27-hydroxylase (CYP27A1) that is expressed throughout the body (Heverin et al., 2005; Uhlén et al., 2015) and the majority of 27-OH in the brain has its origin outside the central nervous system (Heverin et al., 2005). 27-OH fluxes into the brain in a concentration-dependent manner where the concentrations are higher in white than gray matter (Heverin et al., 2005). 27-OH concentrations are relatively low overall, but both brain (Heverin et al., 2004) and cerebrospinal fluid (CSF) (Leoni et al., 2004; Mateos et al., 2011) levels are increased in neurodegenerative diseases compared to healthy controls. In elderly persons at risk of dementia who participated in the lifestyle randomized controlled trial FINGER, higher 27-OH was associated with lower hippocampal volume and cognition (Sandebring-Matton et al., 2021). In preclinical models, the effects of excess brain levels of 27-OH have been linked to reduced dendritic spine density in the hippocampus (Merino-Serrais et al., 2019), reduced neuronal glucose uptake, and impaired memory (Ismail et al., 2017). Further, inflammatory responses by 27-OH including astrocytic activation have been shown (Loera-Valencia et al., 2021; Staurenghi et al., 2021; Testa et al., 2014).

White matter changes are a key factor in lipid dyshomeostasis-associated neurodegenerative diseases and dementia (Bartzokis, 2011; Nasrabady et al., 2018). Changes in white matter integrity emerge in the early stages of neurodegeneration (Lee et al., 2016) which may provide a window of opportunity for new treatment strategies. For instance, in studies conducted on asymptomatic subjects with an increased risk of developing AD, a decline in white matter integrity has been associated with pathological AD biomarker levels (Dean et al., 2017). Despite these discovered associations, the role of myelin and oligodendrocytes in neurodegenerative process remains relatively poorly studied.

27-OH reduces cholesterol synthesis in vitro and in vivo (Ali et al., 2013; Meir et al., 2002), which raises concerns regarding proper myelin assembly and maintenance for which adequate cholesterol levels are essential. Still, to our knowledge, how 27-OH affects oligodendrocytes and myelination-related processes remains unknown. Here, we investigated whether 27-OH influences oligodendrocyte populations and myelin integrity. First, the impact of 27-OH treatment on cell differentiation in cultured oligodendrocytes was studied, whereafter oligodendrocyte gene expression was measured in primary co-cultures. Second, learning and memory of female mice overexpressing sterol 27-hydroxylase (Cyp27Tg) and age-matched non-transgenic littermates (Tg−) were assessed with a battery of cognitive tests, and brain sections from the mice were stained for markers of immature and mature oligodendrocytes, and myelin content. Lastly, associations between 27-OH and myelination proteins in CSF samples from memory clinic patients were analyzed.

KEYWORDS
27-hydroxycholesterol, myelin, neurodegeneration, oligodendrocyte, white matter
2 | MATERIALS AND METHODS

2.1 | Animals

All experimental procedures on mice were performed following the local national animal care and use guidelines of Sweden and Spain and approved by the Swedish Board of Agriculture and Ethics Committees of the University of the Basque Country (UPV/EHU) (ethical permit IDs 4884/2019 and M20/2017/011, respectively). All possible efforts were made to minimize the suffering and distress of the animals.

2.2 | Primary rat oligodendrocyte cultures

For viability assay, oligodendrocytes were obtained from optic nerves of 12-days-old Sprague Dawley rats, as described previously (Barres et al., 1992). Cells were maintained at 37°C and 5% CO2 in a chemically defined medium (OL differentiation medium) composed of Dulbecco’s modified Eagle’s medium ( Gibco) supplemented with 5 μg/mL insulin, 100 μg/mL transferrin, 62.5 ng/mL progesterone, 40 ng/mL sodium selenite, 16 μg/mL putrescine, 1 mg/mL bovine serum albumin (BSA), 63 μg/mL N-acetyl-cysteine, 2 mM L-glutamine (all from Sigma-Aldrich, Burlington, MA, USA), 100 U/mL penicillin/streptomycin (Lonza), 30 ng/mL triiodothyronine, 40 ng/mL thyroxine (both from Sigma-Aldrich) plus 10 ng/mLCNTF and 1 ng/mL NT3 (both from Peprotech, Cranbury, NJ, USA). After 1–2 days in vitro, cultures were composed of at least 98% O4+ GaIC+ cells; the majority of the remaining cells were glial fibrillary acidic protein (GFAP) positive. Microglial cells were not detected in these cultures (Quintela-López et al., 2019).

For maturation analysis, primary cultures of oligodendrocyte precursors cells (OPCs) were prepared from mixed glial cultures obtained from newborn (P0–P2) Sprague Dawley rat forebrain cortices as previously described (McCarthy & De Vellis, 1980) with minor modifications. Briefly, forebrains were removed from the skulls and the cortices were isolated and enzymatically digested by incubation with 0.25% trypsin and 4% DNase for 15 min at 37°C. Then, the tissue was mechanically dissociated and plated in Iscove’s Modified Dulbecco’s Medium (IMDM) supplemented with 10% fetal bovine serum (PBS). Hyclone). The mixed glial cells were grown in T75 flasks (pre-treated with poly-D-lysine) until they were confluent (10–12 days). Microglia were separated from the cultures by shaking the flasks on a rotary shaker for 1 h at 250 revolutions/min. OPCs were isolated following an additional 18 h. OPCs were seeded onto poly-D-lysine-coated coverslips and were maintained at 37°C and 5% CO2 for 3 days in vitro (DIV) in an OL differentiation medium. The purity of the oligodendroglial cultures was routinely assessed by examining the characteristic cell morphologies under phase-contrast microscopy and was confirmed by immunostaining with cell-type specific antibodies against Olig2 (AB15328, 1:1000, Millipore, MA, USA), PDGFR (MAB3402, 1:200, Santa Cruz Biotechnology, CA, USA), GFAP (MAB3402, 1:80, Millipore), or Iba1 (019–19741, 1:2000, Wako, Madison, WI, USA). After 2 days in culture Olig2+ (Oligodendrocyte transcription factor 2) cells represented 95% ± 0.2% and PDGFR+ (Platelet-derived growth factor receptor α) oligodendrocyte progenitors represented 92.5% ± 0.5% of total cells (Bernal-Chico et al., 2015).

2.3 | Cell viability assay

Cell viability assays in primary oligodendrocytes were performed with Calcein AM Cell Viability Assay (Life Technologies, Carlsbad, CA, USA). Briefly, cells were seeded into 24-well plates bearing 12-mm-diameter coverslips coated with poly-D-lysine (10 μg/mL) at a density of 1 × 10⁶ cells per well. After 1 day in vitro, cultured oligodendrocytes were treated with increasing concentrations of DMSO or 27-OH (0.1–10 μM, Avanti Polar Lipids, Alabaster, AL, USA) for 24 h. Cultured oligodendrocytes were then incubated with Calcein AM at 1 μM and 37°C for 30 min in fresh culture medium and thereafter washed in pre-warmed 0.1 M PBS three times. Emitted fluorescence was measured by a Synergy HT (Biotek, Winooski, Vermont, USA) spectrophotometer using excitation wavelength at 485 and emission at 528 nm.

2.4 | Immunocytochemistry of cultured oligodendrocytes

Cells were seeded into 48-well plates coated with poly-D-lysine (10 μg/mL) at a density of 2 × 10⁵ cells per well. For O4 antigen immunostaining, live cells were incubated 30 min at 37°C with mouse anti-O4 (MAB1326, 1:100, R&D systems, Minneapolis, MN, USA) in OL differentiation medium. Then, cells were fixed with 4% paraformaldehyde and 2% saccharose in PBS for 15 min at room temperature (RT) and rinsed three times with PBS. Cells were thereafter permeabilized and blocked in 4% normal goat serum (NGS), 0.1% Triton X-100 in PBS (blocking buffer) for 1 h and incubated overnight at 4°C with primary antibodies against mouse anti-MBP (SMI 99, 1:1000, Biologic, San Diego, CA, USA) and mouse anti-Olig2 (MABN50, 1:1000, Millipore). Cells were washed in PBS and incubated with fluorophore-conjugated Alexa secondary antibodies (1:500) in a blocking buffer for 1 h at RT. Cell nuclei were detected by incubation with DAPI (4 μg/mL, Sigma-Aldrich). Samples were mounted with Fluoromount-G (Southern Biotech, Birmingham, AL, USA). The analysis was done automatically using the HighContent Imaging system (Thermo Fisher Scientific, Waltham, MA, USA). Image acquisition was determined on the center fields for all the wells. Olig2+, O4+, and MBP+ cells were counted automatically from 10 fields per coverslip using a 20× objective using a High Content Imaging system.

2.5 | Primary co-cultures

For cell co-cultures wild-type, C57BL/J6 mice were used. Female mice were let to breed with males overnight to ensure accurate embryonic day (E) of the embryos used for primary cell cultures. At E16–E17, the embryos were sacrificed by decapitation, heads of the...
embryos were collected and, kept in Hibernate™-E Medium (Gibco) until the dissection of brains. Dissection was performed in EBSS solution (Life Technologies) and only dissected hippocampus and cortex were used for the co-culture set-up. To extract the cells, tissues were first incubated at 37°C in a 1:1 mix of solution A (0.0625% trypsinization solution [Life Technologies] in EBSS) and solution B (0.08 mg/mL DNAse1 solution [Roche, Basel, Schweiz] in EBSS) for approximately 15 min gently moving. Trypsinization was stopped with culture media containing 2% B27 supplement (Gibco), 1% N2 supplement (Gibco), 100 units/mL penicillin and 100 μg/mL streptomycin (Thermo Fisher Scientific), in DMEM/F-12 + GlutaMAX (Gibco). After supernatant removal, the tissue was mechanically disintegrated by pipetting up and down in culture media. The supernatant containing the cells was collected and centrifuged at 500 μL droplet with Matrigel in a ratio of 1:1. Droplets were let settle for 1 h before adding 1 mL of the culture media. Cultures were treated at DIV1 with 1μM 27-OH or 5.6 mM 27-OH ranges approximately from 1 to 6 ng/mg both in humans and mice (Ali et al., 2013; Heverin et al., 2004; Testa et al., 2016). For this study, 8-month-old female Cyp27tg mice and their age-matched non-transgenic littermates (Tg–) were used. The mice were fed normal chow and water was provided ad libitum. For behavioral analyses we studied two separate cohorts: for Morris Water Maze (MWM), we tested 10 transgenic and 10 non-transgenic mice, and for Y-maze and Fear Conditioning (FC) nine transgenic and five non-transgenic mice. Brain tissues from Cyp27tg and Tg– were dissected and used for biochemical analyses.

2.8 | Behavioral tests

For MWM, each mouse was tested for four trials per day, for five consecutive days. Reference memory was evaluated on the sixth day (probe test). MWM, Y-maze, and the FC tests were performed as described previously (Eroli et al., 2020; Maioli et al., 2013) except that for FC the conditional stimulus of sound was on day one where mice were exposed to a 55 dB sound at 5000 Hz for 30 s followed by a mild foot shock (0.3 mA for 2 s). The sound-shock pairing was repeated three times in total with a 50-s interval between each one. Cue FC was performed on day three, where mice were let to explore the new surrounding for 2 min, whereafter the sound (55 dB at 5000 Hz) lasted for 2 min continuously. The rectangular-shaped chamber was replaced with a round-shaped chamber (20 cm diameter x 35 cm high), and the stainless-steel grid floor was replaced with a black flat surface. Instead of wiping the chamber with ethanol, the chambers were cleaned between each mouse with hypochlorous water (50% dil). Freezing behavior was defined as the complete absence of motility within the same area for 2 s or longer and was measured through TSE Multi Conditioning software. The freezing % recorded during the habituation phase of day 1 (as a measure of baseline freezing) was compared to the freezing % of day 2 to evaluate the context memory. To assess cue memory, we measured the freezing % on day 3 before and during the sound stimulus.

2.9 | Luxol blue staining of mouse brain sections

Paraffin-embedded mouse coronal brain sections of adult Cyp27tg mice and Tg– mice were deparaffinized by serial incubations in xylene or Histolab-Clear (HistoLab, Gothenburg, Sweden) and ethanol (EtOH) of decreasing concentrations (xylene or Histolab-Clear: 2× 10 min, 99.5% EtOH: 2× 5–10 min, 95% EtOH: 1× 3–5 min, 70% EtOH: 1× 3–5 min), after which they were washed in deionized water. Luxol Fast Blue Stain Kit (Abcam, Cambridge, UK) was used according to the protocol of the manufacturer. Briefly, brain sections were incubated in Luxol Fast Blue (LFB) for 2 h at 60°C, rinsed thoroughly with distilled H2O, and dipped in lithium carbonate for differentiation.
Differentiation was continued by dipping the slides into 70% alcohol reagent until the gray matter was colorless. Then slides were quickly rinsed with distilled H2O, dehydrated with three changes in 99.5% ethanol, and mounted on coverslips with Vecta Mount mounting media. Images were acquired with Nikon Eclipse E800 light microscope, using a 10× objective, coupled to a Nikon DS-Ri2 camera and processed with NIS-Elements imaging software (version 4.30.00).

Blinded analysis of LFB stained sections was performed using ImageJ 1.52p (Schindelin et al., 2012) as described previously (Khodanovich et al., 2017; Underhill et al., 2011). The mean intensity of each brain section was measured from the corpus callosum and fimbria. Mean intensities of the red channel (I_R) in regions of interest were measured from RGB images of LFB stained sections without cresyl echt violet stain. Similarly, the mean intensity of the background (I_B) was measured in each image. Optical densities (%) of each region were calculated as $100 \times (1 - [I_R/I_B])$, resulting in higher myelin content with increasing value.

### 2.10 Immunofluorescence staining of mouse brain sections

Deparaffinized sections were subjected to heat-induced treatment (121°C for 20 min) in R-Universal buffer (Aptum, Southampton, UK) for epitope recovery. The sections used for CC1 and Olig2 staining were washed with cold PBS and treated with ice-cold 100% EtOH for 10 min at -20°C. This step was not performed on the sections stained for PDGFRα, MBP, myelin oligodendrocyte glycoprotein (MOG), and enzyme 2’3’-Cyclic-nucleotide 3’-phosphodiesterase (CNPase). The sections were blocked for 1 h at RT in a blocking buffer with 0.1% Triton X-100, and 10% NGS (Vector Laboratories, Burlingame, CA, USA) in PBS for 1 h, and incubated overnight at 4°C with primary antibodies. Slices were washed in PBS and incubated with fluorophore-conjugated Alexa secondary antibody (1:500) in blocking buffer for 1 h at RT. Finally, the sections were treated with an auto-fluorescence eliminator reagent (Millipore) for 5 min to reduce auto-fluorescence and mounted with SlowFade™ Gold Antifade (Thermo Fisher Scientific) or Fluoromount-G (SouthernBiotech).

Primary antibodies used to acquire Figures 3e and 4 were chicken anti-MBP (AB9348, 1:200, Millipore), mouse anti-MOG (MAB5680, 1:200, Millipore), mouse anti-CNPase (C5922, 1:500, Sigma-Aldrich), mouse anti-CC1 (OP80, 1:200, Millipore), and mouse anti-Olig2 (MABN50, 1:1000, Millipore). Images were acquired with Leica TCS STED SP8 laser scanning confocal microscope using a 40× oil objective and analysis was carried out in two sections per subject, taking three different pictures for each region. Images were taken with the same setting for all experiments. For MBP, MOG, and CNPase analysis, mean intensity and area values were quantified from the corpus callosum and cortex with Image J software. For CC1/Olig2 analysis, the percentage was calculated as the ratio of CC1+Olig2+/Olig2+ cells considering the total number of Olig2 cells as a 100% value.

Images for Figure 3d were acquired from sections co-incubated with rat anti-PDGFRα (562777, 1:1000, BD Biosciences, Franklin Lakes, NJ, USA) together with anti-NeuN (ABN78, Sigma-Aldrich) in incubation buffer (0.3% Triton X-100, 10% NGS, 1% BSA) overnight at 4°C. Sections were incubated with goat anti-rat Alexa Fluor 488 1:300 (A11006, Invitrogen) and anti-NeuN and DAPI 1:1000 secondary antibodies for 2 h at RT and protected from light. The stained tissue was analyzed using a confocal microscope (Zeiss LSM-800 Airy system) with a 20× objective. Three pictures from each region, corpus callosum, cortex, and hippocampus, were captured from one section of each mouse using the Zen software (ZEISS Microscopy, Jena, Germany). Imaging settings were kept constant for each staining type. In a blinded experiment, the number of PDGFRα positive cells was quantified for each region (total area of approximately 918 mm²/region) by picture.

### 2.11 Preparation of myelin-enriched fractions

Dissected brains were removed from the meninges, choroid plexus, cerebellum, and brainstem and minced in DMEM. Brain homogenates were incubated with 2.5 mg/mL DNAse I and 2.5 mg/mL Trypsin/EDTA for 30 min–1 h at 37°C on an orbital shaker at 180 rpm for tissue digestion. Thereafter, tissue suspension was mixed with DMEM and centrifuged at 1000×g for 10 min at 4°C. Pellet was then resuspended in 20% w/v BSA-DMEM and centrifuged 1000×g for 20 min at 4°C. The upper layer (myelin) was collected using a Pasteur pipette.

### 2.12 Immunoblotting of brain lysates and cerebrospinal fluid samples

Myelin enriched fractions were homogenated with a douncer in RIPA buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 1% NPO-40 in 0.1 M PBS) supplemented with protease inhibitor cocktails (Roche). Afterward, they were sonicated for 25 cycles at 80% amplitude (Labsonic M, Sartorius, Göttingen, Germany), centrifuged 2000×g for 10 min at 4°C, and the supernatants were collected. Total protein content was quantified through Bradford assay (Bio-Rad, Hercules, CA, USA) and all the samples were brought to the same concentration. Samples were mixed with 1× sample buffer (62.5 mM Tris pH 6.8, 10% glycerol, 2% SDS, 0.002% bromophenol blue, and 5.7% β-mercaptoethanol in dH2O) and boiled at 95°C for 8 min. Myelin enriched fractions (20 μg) and CSF (11.25 μL) were size-separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 4%–20% Criterion TGX Prestac gels and transferred to nitrocellulose membranes using a Trans-Blot Turbo Transfer System (Bio-Rad). Membranes were blocked in 5% BSA in Tris-buffered saline/0.05% Tween-20 (TBS-T) and proteins detected by specific primary antibodies against MBP (SMI 99, 1:1000; Biolegend), PLP (MAB388, 1:1000, Millipore), MOG (MAB5680, 1:1000, Millipore), CNPase (C5922, 1:1000, Sigma-Aldrich), and β-actin (A20066, 1:5000; Sigma-Aldrich).
2.13 | CSF sampling and clinical biomarkers

CSF samples from memory clinic patients were from the biobank and database GEDOC at Karolinska University Hospital in Sweden. All participants gave written informed consent and permissions from the Swedish Ethical Review Authority to conduct the research have been approved (2011/1987-31/4 and 2019-06056). Samples were collected by standard lumbar puncture between the L3/L4 or L4/L5 intervertebral space using a 25-gauge needle. CSF was aliquoted in polypropylene tubes and stored at −80 °C until further analysis. CSF Aβ42, t-Tau, and p-Tau concentrations were measured on fresh samples with commercially available sandwich enzyme-linked immunosorbent assays (Innogenetics, Ghent, Belgium) according to standardized protocols in the clinic. 27-OH levels were quantified in cerebrospinal fluid using isotope dilution mass spectrometry as described previously (Dzeletovic et al., 1995).

2.14 | Statistical analysis

Analyses were carried out using Prism (version 9.3.1, GraphPad, San Diego, CA, USA), except for CSF data which were analyzed using Stata software (version IC 16.1, StataCorp LLC, College Station, Texas, USA). Results are expressed as mean values and standard deviations. Data obtained from cell and animal studies were analyzed with a two-tailed t-test, Mann–Whitney two-tailed U test, one-way ANOVA, or repeated-measures ANOVA when appropriate. In the case of ANOVA, adjusted p-values are reported. Normality of data was assessed with the Shapiro–Wilk test, and for the CSF data with skewness and kurtosis tests for normality. Zero-skewness log transformation was applied to skewed variables. For the analysis of associations of CSF data, four linear regression models were applied. In Model 1 adjustment was made for age and sex. In Model 2 adjustment was made for age, sex, and Aβ42. In Model 3 adjustment was made for age, sex, and t-Tau. In Model 4 adjustment was made for age, sex, and p-Tau. In all models, myelin proteins were considered dependent variables, and 27-OH as an independent variable. In all analyses, a p value of <.05 was considered significant.

3 | RESULTS

3.1 | 27-OH treatment exhibits toxicity in immature oligodendrocytes and promotes differentiation in oligodendrocyte progenitor cells

We first examined the effect of 27-OH on viability in cultured oligodendrocytes derived from optic nerves. Cell viability significantly decreased with increasing concentrations of 27-OH from 0.5 μM (91% of control) up to 10 μM (80% of control) (Figure 1a). The differentiation stages of oligodendroglia are determined by the expression of lineage-specific cell markers. While Olig2 is maintained throughout all the oligodendroglial lineage, O4 expression begins in late progenitors, and immature and mature oligodendrocytes express MBP (Marques et al., 2016). After 48 h of 1 μM 27-OH treatment, oligodendrocyte differentiation was analyzed by immunocytochemistry for the aforementioned stage-specific cell antigens. Data showed that the percentage of Olig2+/O4−/MBP+ cells was significantly increased with 27-OH treatment when compared with vehicle-treated cells (20.7% vs. 5.6%, respectively, p = .009), suggesting enhanced maturation of 27-OH-treated oligodendrocytes (Figure 1b,c). The number of immature oligodendrocytes Olig2+/O4−/MBP− and Olig2+/O4+/MBP+ were similar between 27-OH and vehicle treatments (p = .839 and p = .296, respectively).

3.2 | 27-OH alters oligodendrocyte gene expression

We next sought to determine the effects on myelin gene expression from 27-OH in mouse co-cultures. Previous data have shown that Olig2 expression is reduced as the oligodendroglial lineage becomes differentiated (Marques et al., 2016). In our cultures in control conditions, Olig2 expression maintains a basal level from DIV3 to DIV5 but decreases by DIV10 (mean difference 0.7 ± 0.2, p = .003, Figure 1d); whereas 27-OH treated cultures show decreased Olig2 expression at DIV3 and DIV5 and not at DIV10 compared to controls (fold change mean differences 0.7 ± 0.2, p = .004, 1.2 ± 0.2 p < .0001, and 0.1 ± 0.2, p = .797, Figure 1d). An opposing expression pattern of the marker of myelinating oligodendrocytes, Cc1, was observed in the same experimental conditions (Figure 1e). Cc1 expression exhibited a difference of 5.0 ± 0.8 in means between 27-OH and vehicle-treated cultures on DIV3 (p < .0001). The difference remained significant at DIV5, yet the magnitude of the difference had decreased to 3.0 ± 0.7 (p = .0005). However, by DIV10 the expression level in 27-OH treated cells had attenuated to a level significantly below the control (mean difference 1.5 ± 0.7, p = .042). The expression of Cc1 was significantly different between DIV5 and 10 in vehicle-treated cultures (1.6 ± 0.7, p = .032). The levels were otherwise stable throughout time points. In 27-OH treated cultures, there was a major reduction in expression from DIV3 to DIV5 (mean difference 2.4 ± 0.8, p = .005), and further from DIV5 to DIV10 (mean difference 2.9 ± 0.7, p = .0007).

Next, we compared the expression of OPC marker Pdgfra between 27-OH treated and untreated cells. Pdgfra was reduced by 27-OH treatment at all studied time points (Figure 1f). At DIV3, the reduction in fold change means was 0.8 ± 0.3 (p = .007) on DIV5 the reduction attenuated to 0.6 ± 0.2 (p = .012) yet remained until DIV10 (0.7 ± 0.2, p = .007). Altogether, our results show that 27-OH changes the dynamics of oligodendrocyte maturation towards a more mature state; a decrease in Olig2 and Pdgfra expression, and an increase in Cc1 expression that promote oligodendrocyte differentiation.

3.3 | Elevated levels of 27-OH result in impairment of spatial learning in female Cyp27 transgenic mice

To mimic long-term hypercholesterolemia, we further investigated the chronic effects of 27-OH on myelin and cognition. As a first step, we
Effects of 27-hydroxycholesterol (27-OH) on oligodendrocyte lineage cells in vitro. (a) Cell viability of primary oligodendrocytes derived from rat optic nerves (98% O4+/GalC+ cells) was assessed with increasing concentrations of 27-OH. The dashed line represents the control treatment value. (b) Immunofluorescence staining of primary oligodendrocytes derived from mixed glial cultures after treatment with 1 μM 27-OH and vehicle for 48 h. Cells were stained for oligodendrocyte transcription factor 2 (Olig2, blue), O4 (green), and myelin basic protein (MBP, red) to identify oligodendrocytes in different maturation stages. White stars indicate O4+ and MBP+ cells and scale bars represent 100 μm. (c) Quantification of positive cells for the different markers: Olig2/O4 and Olig2/MBP. Bars show the percentage of cells labeled with Olig2/O4/MBP antibodies of Olig2+ cells (100%). (d-f) In co-cultures set up from wild-type embryonic mouse hippocampus and cortex containing glial cells and neurons, effects of 1 μM 27-OH treatment were investigated on days-in-vitro (DIV) 3, 5, and 10. In all experiments, cultures were treated at DIV1 with 27-OH or vehicle. (d) To evaluate oligodendrocyte survival, gene expression of Olig2 was compared to the expression of Gapdh. (e) Differences in Cc1 (adenomatous polyposis coli clone 1, a marker for mature oligodendrocytes) and (f) Pdgfra (platelet-derived growth factor receptor α, a marker for immature oligodendrocytes) expression in relation to Olig2. Statistical evaluation was performed using unpaired Student's two-tailed t test (a, c) or one-way ANOVA with uncorrected Fisher's LSD post hoc test (d-f). All data are presented as mean ± SD, *p < .05; **p < .01; ***p < .001.
applied a battery of memory tests on 8-month-old female Cyp27Tg mice and their age-matched non-transgenic littermates (Tg−) to test spatial and fear-associated contextual learning and memory. Memory impairment in Cyp27Tg mice has previously only been reported in male mice (Ismail et al., 2017; Merino-Serrais et al., 2019). In the MWM, the latency for finding the platform significantly decreased in control mice from day one and two, up to day five (mean difference 31.0 s, \( p = .027 \) and 22.7 s, \( p = .040 \), respectively; Figure 2a), indicating that the control group was able to learn the task. However, learning was not seen in Cyp27Tg mice, where there were no statistically significant differences observed in escape latency between training days. When comparing the groups during the learning phase, control mice were 23.4 s faster to find the platform than Cyp27Tg mice on the last training day (\( p = .054 \), Figure 2a). The speed (cm/s) did not differ between strains (Cyp27Tg 18.1 cm/s, control 19.9 cm/s, \( p = .143 \), Figure 2b) excluding the possibility of differences observed in learning to be a result of lack in motivation or differences in motor function. On the 6th day (probe test) when the platform was removed, mice from both strains spent a similar amount of time in the target zone (Cyp27Tg 12.6 s, Tg−/C0 15.6 s, \( p = .356 \), Figure 2c).

In Y-maze, there was no significant difference between the percentage in alternation between arms (Cyp27Tg 58.5%, Tg− 62.5%,
Neither was there a significant difference between the number of entries into the different arms between strains (Cyp27Tg 20.1, Tg – 14.2, \( p = .063 \), Figure 2d). On the first day of FC mice were exposed to a high-frequency sound and an electric shock after the sound. On the second day, the floor was kept as on day 1 (context), but mice were not exposed to sound or electrical...
FIGURE 4 Chronic exposure to 27-hydroxycholesterol (27-OH) increased myelin basic protein (MBP) levels in the corpus callosum and cerebral cortex.

(a) The image shows the areas analyzed in the cortex and corpus callosum (marked with white squares). (b) MBP, (c) MOG, and (d) CNPase stainings in Tg− and Cyp27Tg brains. Graphs show the intensity measures of (e) MBP, (f) MOG, and (g) CNPase stainings in the corpus callosum. (h) MBP, (i) MOG, and (j) CNPase positive areas in the cortex. Statistical evaluation was performed using unpaired Student’s two-tailed t test. All data are presented as mean ± SD, *p < .05.
shock. In both strains, the time freezing increased significantly on the second day (Cyp27Tg 29.9%, Tg/C0 41.9%, for both \( p < .0001 \), Figure 2f). However, the amount of freezing during the second day was less for Cyp27Tg than for Tg/C0 mice, yet the difference was not significant (\( p = .074 \)). On the third day, mice were exposed to the same sound (cue) as on the first day on a new floor (change of context). Both strains increased their freezing time significantly during the sound compared to the time before the sound (Cyp27Tg 47.2% \( p < .0001 \), Tg/C0 37.3% \( p = .001 \), Figure 2g). There was no significant difference between strains in freezing time before sound (\( p = .103 \)) nor during sound (\( p = .782 \)).

### 3.4 Chronic exposure to 27-OH in vivo does not alter myelin overall structure but increases MBP levels

To assess the overall myelin status in the Tg– and Cyp27Tg mice, brain sections were stained with LFB. Myelin in the corpus callosum at the age of 8 months did not differ between strains (\( p = .427 \), Figure 3a,b). Neither was there a difference in LFB staining of fimbria between strains (\( p = .108 \), Figure 3a,c). To investigate differentiation stages of oligodendrocytes and myelin protein levels in Cyp27Tg brains, brain sections were stained for PDGFR\( \alpha \), CC1/Olig2, MBP, MOG, and CNPase. Chronic exposure to 27-OH did not have an impact on the PDGFR\( \alpha \) positive cells at the age of 8 months (Figure 3d,f), however, the number of CC1/Olig2-positive cells increased in the cortex but not in the corpus callosum or hippocampus of transgenic mice compared to non-transgenic mice (89.35% vs. 68.46% of total number of Olig2\(^+\) cells, \( p = .008 \), Figure 3e,g). Immunohistochemistry analysis of myelin proteins showed that the MBP mean intensity was significantly increased in the corpus callosum of Cyp27Tg (\( p = .037 \), Figure 4b,e). Moreover, the MBP-positive cortical area was increased in the transgenic mice by 3.7% (\( p = .049 \), Figure 4b,h). Still, MOG (Figure 4c) and CNPase (Figure 4d) expression in the corpus callosum and in the cortical area did not differ between the strains (\( p = .233 \) Figure 4f and \( p = .168 \) Figure 4i; \( p = .899 \) Figure 4g and \( p = .867 \) Figure 4j, respectively). In myelin-enriched fractions of brain homogenates, MBP levels were increased in Cyp27Tg mice (\( p = .030 \) Figure 5a,b). Conversely, levels of MOG and proteolipid protein (PLP) were not significantly different between lines (\( p = .059 \), Figure 5a,c and \( p = .076 \), Figure 5a,d).
3.5 | 27-OH is associated with CNPase levels in CSF from memory clinic patients

To determine if our findings could be reflected in human samples, we subsequently measured 27-OH, MBP, and CNPase in CSF from a cohort of memory clinic patients with a diagnosis of subjective cognitive impairment (SCI), mild cognitive impairment (MCI), or AD. Demographics, clinical characteristics, and mean biomarker levels of the cohort are presented in Table 1. Results from the linear regression analyses are presented in Table 2. We did not observe any statistically significant associations between MBP and 27-OH in any of the models suggesting that myelin is neither degraded nor released from the brain in a 27-OH concentration dependent manner. The myelination regulating enzyme CNPase was instead associated significantly with 27-OH in age and sex-adjusted Model 1 ($\beta = 0.38$, $p = .022$) and the significance remained after further adjustment for the AD-related pathologies: $A_{\beta}42$, t-Tau, and p-Tau levels (Model 2, 3, and 4 respectively). The results did not change even if models were further adjusted for cognition (data not shown). As expected, the CSF levels of myelin proteins MBP and CNPase were associated with each other (Model 1: $\beta = 0.70$, $p < .0001$). The myelin protein levels did not associate with the levels of AD biomarkers, except for MBP which was associated with $A_{\beta}42$ ($\beta = 0.32$, $p = .034$). Neither of the myelin proteins was associated with cognition as measured by RAVLT or MMSE when adjusted for age, sex, and education.

4 | DISCUSSION

Cholesterol and its metabolites are fundamental in the physiology of the nervous system and consequently, peripheral hypercholesterolemia is a condition that has been associated with brain disorders and cognitive deficits (Anstey et al., 2008). Changes in the white matter are an early phenomenon in cognitive disorders and are linked to vascular factors including high cholesterol (Murray et al., 2005). Previous studies show that the BBB passing cholesterol metabolite 27-OH is associated with changes in neuronal morphology and function affecting cognition, and that also astrocytes are influenced (Loera-Valencia et al., 2019). Nevertheless, its effects on the white matter are unknown. This study aimed to investigate the specific effects of 27-OH on oligodendrocytes and myelin. We found that 27-OH is slightly toxic in cultured oligodendrocytes, but also that it alters the maturation pattern in both cell cultures and in adult Cyp27Tg mice, which chronically express high 27-OH levels. Further, in CSF samples from a memory clinic cohort, we show a positive association between 27-OH and a myelination-regulating enzyme CNPase. Our findings suggest that oligodendrocytes are influenced by 27-OH in a way promoting their maturation which may have implications for hypercholesterolemia-associated changes in the white matter.

The establishment of oligodendrocyte maturity and proper function is an intricate process regulated by the expression of multiple genes (Liu et al., 2019; Yu et al., 2013). We showed that 27-OH influences $Olig2$, $Cc1$, and $Pdgfra$ expression in oligodendrocyte cultures and thereby promotes the maturation process. This was seen even in co-cultures where the presence of other brain cells could buffer its effect through CYP27A1 and CYP7B1 metabolism (Loera-Valencia et al., 2019). In the Cyp27Tg in vivo model, the changes were not as prominent as in vitro; the number of PDGFRa-positive OPCs was on the same level between Cyp27Tg and Tg- mice yet the numbers of CC1-expressing oligodendrocytes increased in the cortex but not in hippocampus or corpus callosum.

Interestingly, we detected a consequent increase in levels of the late myelin marker MBP in both cell cultures treated with 27-OH and in the cortex and corpus callosum of adult mice over-expressing 27-OH. MBP is not only affecting myelin compaction (Snaidero et al., 2017) but it is also one of the rate-limiting myelin formation factors together with e.g. cholesterol and CNPase (Lappe-Siefke et al., 2003; Popko et al., 1987; Saher et al., 2005). Further, altering MBP may affect the intricate interplay between a proper lipid composition and protein profile that is crucial for myelin homeostasis and function (Krugmann et al., 2020). Whether these 27-OH-induced MBP changes are altering overall myelin structure and function remain to be determined, yet our LFB staining did not indicate any major structural changes in the Cyp27Tg mice suggesting that the changes are subtle.

Previous studies show that male Cyp27Tg mice exhibit deficits in memory at the age of 8–9 months along with impaired neuronal morphology and function (Ismail et al., 2017; Merino-Serrais et al., 2019). In mice modeling cerebrovascular pathology, high-cholesterol-diet induced white matter changes and reduced numbers of immature oligodendrocytes, accompanied by cognitive deficits (Tong et al., 2019). In our study, female Cyp27Tg mice displayed impairment in learning in MWM at the age of 8 months, but not in Y-maze and FC tests,
indicating sex differences in spatial learning. The findings of the current study unveil an effect of 27-OH in oligodendroglia and myelin which act in concert with previously described 27-OH-induced changes (Loera-Valencia et al., 2019) ultimately resulting in impaired cognition.

Myelin proteins are released into CSF when the white and gray matter is damaged (Sellebjerg et al., 2017). In post-mortem brains of AD patients, there is a depletion of cholesterol along with a reduction in MBP and CNPase (Roher et al., 2002). Moreover, 27-OH levels in CSF correlate with brain structural damage in diseases including demyelinating polyneuropathy and AD (Leoni et al., 2002; Mateos et al., 2011), emphasizing a plausible connection between this oxysterol in CSF and demyelination and/or neuronal damage. Notably, increased CSF 27-OH levels correlate with white matter hyperintensities in a memory clinic cohort (Besga et al., 2012) and in cases of type 5 spastic paraplegia, a disease that results in elevated 27-OH levels due to mutations in the CYP7B1 gene that is responsible for catabolizing excess 27-OH (Biancheri et al., 2009). Interestingly, in CSF from a memory clinic cohort we found a positive association between CNPase and 27-OH that was independent of clinical AD biomarker levels. Based on the available literature (Raasakka & Kursula, 2014) CNPase is important for the formation of myelin membranes and in maintaining axonal integrity. Whether the associations between 27-OH and CNPase in CSF reflect oligodendrocyte maturation in the human brain should be further investigated in larger cohorts of individuals with elevated 27-OH levels.

In sum, we found that 27-OH is toxic to immature oligodendrocytes while favoring their OPC maturation and modifying protein levels in myelin. These features may underlie part of the effects of hypercholesterolemia on brain function, yet the clinical implications of these findings should be further evaluated.

AUTHOR CONTRIBUTIONS
Angel Cedazo-Minguez, Carlos Matute, Elena Alberdi, and Anna Sandebring-Matton: designed the research project. Vilma Alanko, Adhara Gaminde-Blasco, Tania Quintela-López, Ingemar Björkhem, Graziella Tabacaru, María Latorre-Leal: performed and analyzed experiments with input from Alina Solomon, Angel Cedazo-Minguez, Raúl Loera-Valencia, Carlos Matute, Silvia Maioli, Mía Kivipelto, Elena Alberdi, and Anna Sandebring-Matton. Vilma Alanko: drafted the first version of the manuscript with input from Adhara Gaminde-Blasco, Anna Sandebring-Matton, Carlos Matute, and Elena Alberdi. All authors read, reviewed, edited, and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
The data generated and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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REFERENCES
Ali, Z., Heverin, M., Olin, M., Acimovic, J., Lövgren-Sandblom, A., Shafati, M., Bävner, A., Meiner, V., Leitersdorf, E., & Björkhem, I. (2013). On the regulatory role of side-chain hydroxylated oxysterols in the brain. Lessons from CYP27A1 transgenic and Cyp27a1(−/−) mice. Journal of Lipid Research, 54(4), 1033–1043. https://doi.org/10.1194/jlr.M034124
Anstey, K. J., Ashby-Mitchell, K., & Peters, R. (2017). Updating the evidence on the association between serum cholesterol and risk of late-life dementia: Review and meta-analysis. Journal of Alzheimer’s Disease, 56(1), 215–228. https://doi.org/10.3233/JAD-160826
Anstey, K. J., Lipnicki, D. M., & Low, L. F. (2008). Cholesterol as a risk factor for dementia and cognitive decline: A systematic review of prospective studies with meta-analysis. American Journal of Geriatric Psychiatry, 16(5), 343–354. https://doi.org/10.1097/JGP.0b013e3181761941
Appleton, J. P., Scott, P., Sprigg, N., & Bath, P. M. (2017). Hypercholesterolaemia and vascular dementia. Clinical Science, 131(14), 1561–1578. https://doi.org/10.4265/CS20160382
Barres, B. A., Hart, I. K., Coles, H. S. R., Burme, J. F., Vovodic, J. T., Richardson, W. D., & Raff, M. C. (1992). Cell death and control of cell survival in the oligodendrocyte lineage. Cell, 70(1), 31–46. https://doi.org/10.1016/0092-8674(92)90531-G
Bartozkoski, G. (2011). Alzheimer’s disease as homeostatic responses to age-related myelin breakdown. Neurobiology of Aging, 32(8), 1341–1371. https://doi.org/10.1016/j.neurobiolaging.2009.08.007
Bernal-Chico, A., Canedo, M., Manterola, A., Victoria Sánchez-Gómez, M., Pérez-Samartín, A., Rodriguez-Puertas, R., Matute, C., & Mato, S. (2015). Blockade of monoacylglycerol lipase inhibits oligodendrocyte...
excitotoxicity and prevents demyelination in vivo. Glia, 63(1), 163–176. https://doi.org/10.1002/GLIA.22742

Besga, A., Cedazo-Minguez, A., Kärnell, H., Solomon, A., Björkhem, I., Winblad, B., Leoni, V., Hooshmand, S., Spulber, G., Gonzalez-Pinto, A., Kivipelto, M., & Wahlund, L. O. (2012). Differences in brain cholesterol metabolism and insulin in two subgroups of patients with different CSF biomarkers but similar white matter lesions suggest different pathogenic mechanisms. *Neuroscience Letters*, 510(2), 126. https://doi.org/10.1016/j.neulet.2012.01.017

Blancheri, R., Ciccolella, M., Rossi, A., Tessa, A., Cassandrini, D., Minetti, C., & Santorelli, F. M. (2009). White matter lesions in spastic paraplegia with mutations in SPG/CYP7B1. *Neuro muscular Disorders*, 19(1), 62–65. https://doi.org/10.1016/j.nmd.2008.10.009

Björkhem, I., Heverin, M., Leoni, V., Meaney, S., & Diczfalusy, U. G. (2006). The Journal of Experimental Medicine, 207(3), 895–899. https://doi.org/10.1084/jem.20060287

Björkhem, I., Lütjohann, D., Bayer, T., Pikuleva, I., Hilmer, S., Wastesson, J. W., de Bem, A. F., Krolow, R., Farias, H. R., de Rezende, V. L., Gelain, D. P., Björkhem, I., Leoni, V., Meaney, S., & Diczfalusy, U. G. (2004). Diagnostic use of cerebro and extracerebral oxysterols. *Clinical Chemistry and Laboratory Medicine*, 42(2), 186–191. https://doi.org/10.1515/CCLM.2004.034

Lisko, I., Kulkam, J., Annetorp, M., Ngandu, T., Mangialasche, F., & Kivipelto, M. (2020). How can dementia and disability be prevented in older adults? Where are we today and where are we going? *Journal of Internal Medicine*, 289, 807–830. https://doi.org/10.1111/joim.13227

Liu, Z., Yan, M., Liang, Y., Liu, M., Zhang, K., Shao, D., Jiang, R., Li, L., Wang, C., Nussenzveig, D. R., Zhang, K., Chen, S., Zhong, C., Mo, W., Fontoura, B. M. A., & Zhang, L. (2019). Nucleoporin Sel1 interacts with Olig2/Brd7 to promote oligodendrocyte differentiation and myelination. *Neuron*, 102(3), 587–601. https://doi.org/10.1016/j.neuron.2019.02.018

Loera-Ramirez, F., Goikoela, J., Parrado-Fernandez, C., Merino-Serrais, P., & Mañill, S. (2019). Alterations in cholesterol metabolism as a risk factor for developing Alzheimer’s disease: Potential novel targets for treatment. *Journal of Steroid Biochemistry and Molecular Biology*, 190, 104–114. https://doi.org/10.1016/j.jsbmb.2019.03.003

Loera-Ramirez, F., Iñgal, A. A., Goikoela, J., Lodero, M., Mateos, L., Björkhem, I., Puerta, E., Romão, M. A., Gomes, C. M., Merino-Serrais, P., Mañill, S., & Cedazo-Minguez, A. (2021). Hypercholesterolemia and 27-hydroxycholesterol increase S100A8 and RAGE expression in the brain: A link between cholesterol, alarmins, and neurodegeneration. *Molecular Neurobiology*, 58(12), 6063–6076. https://doi.org/10.1007/s12035-021-02521-8

Mañill, S., Bäver, A., Ali, Z., Heverin, M., Iñgal, A. A., Puerta, E., Olín, M., Saeed, A., Shaafati, M., Parini, P., Cedazo-Minguez, A., & Björkhem, I. (2013). Is it possible to improve memory function by upregulation of the 27-hydroxycholesterol 5100A8 and RAGE expression in the brain: A link between cholesterol, alarmins, and neurodegeneration. *Molecular Neurobiology*, 58(12), 6063–6076. https://doi.org/10.1007/s12035-021-02521-8

Marques, S., Zeisel, A., Codeluppi, S., Van Bruggen, D., Falcão, A. M., Xiao, L., Li, H., Häring, M., Hochgener, H., Romanov, R. A., Gyllborg, D., Muñoz-Manchado, A. B., La Manno, G., Lönnerberg, P., Floriddia, E. M., Rezayee, F., Ennors, P., Arenas, E., Hjerling-Leffler, J., Castelo-Branco, G. (2016). Oligodendrocyte heterogeneity in the mouse juvenile and adult central nervous system. *Science*, 352(6291), 1326–1329. https://doi.org/10.1126/science.aaf6463

Mateos, L., Iñgal, A. A., Gil-Bea, F. J., Leoni, V., Winblad, B., Björkhem, I., & Cedazo-Minguez, A. (2011). Upregulation of brain renin angiotensin system by 27-hydroxycholesterol in Alzheimer’s disease. *Journal of Alzheimer’s Disease*, 24(4), 669–679. https://doi.org/10.3233/JAD-2011-101512

McCarthy, K. D., & De Vellis, J. (1980). Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. *Journal of Cell Biology*, 85(3), 890–902. https://doi.org/10.1083/jcb.85.3.890

Meir, K., Kitsberg, D., Alkalay, I., Szafer, F., Rosen, H., Shpitzen, S., Avi, L. B., Stael, B., Fievet, C., Meiner, V., Björkhem, I., & Leitersdor, E. (2002). Human stem 27-hydroxycholesterol (CYP27) overexpressor transgenic mouse
model. Evidence against 27-hydroxycholesterol as a critical regulator of cholesterol homeostasis. *The Journal of Biological Chemistry*, 277(37), 34036–34041. https://doi.org/10.1074/jbc.M201122200

Merino-Serrais, P., Loera-Valencia, R., Rodríguez-Rodríguez, P., Parrado-Fernandez, C., Ismail, M. A., Maioli, S., Matute, E., Jimenez-Mateos, E. M., Björkhem, I., DeFelice, J., & Cedazo-Minguez, A. (2019). 27-hydroxycholesterol induces aberrant morphology and synaptic dysfunction in hippocampal neurons. *Cerebral Cortex*, 29(1), 429–446. https://doi.org/10.1093/cercor/bhy274

Murray, A. D., Staff, R. T., Shenkin, S. D., Deary, I. J., Starr, J. M., & Whalley, L. J. (2005). Brain white matter hyperintensities: Relative importance of vascular risk factors in nondemented elderly people. *Radiology*, 237(1), 251–257. https://doi.org/10.1148/radiol.2371041496

Nasrabady, S. E., Rizvi, B., Goldman, J. E., & Brickman, A. M. (2018). White matter changes in Alzheimer's disease: A focus on myelin and oligodendrocytes. *Acta Neuropathologica Communications*, 6(1), 22. https://doi.org/10.1186/s13195-021-00790-y

Popko, B., Puckett, C., Lai, E., Shine, H. D., Readhead, C., Takahashi, N., Hunt, S. W., III, Sidman, R. L., & Hood, L. (1987). Myelin deficient mice: Expression of myelin basic protein and generation of mice with varying levels of myelin. *Cell*, 48(4), 713–721. https://doi.org/10.1016/0092-8674(87)90249-2

Quintela-López, T., Ortiz-Sanz, C., Serrano-Regal, M. P., Gaminde-Blasco, A., Valero, J., Baierla, J., Sánchez-Gómez, M. V., Matute, C., & Alberdi, E. (2019). Aβ oligomers promote oligodendrocyte differentiation and maturation via integrin β1 and Fyn kinase signaling. *Cell Death & Disease*, 10(6), 1–16. https://doi.org/10.1038/s41419-019-1636-8

Raasakka, A., & Kursula, P. (2014). The myelin membrane-associated enzyme 2′,3′-cyclic nucleotide 3′-phosphodiesterase: On a highway to structure and function. *Neuroscience Bulletin*, 30, 956–966. https://doi.org/10.1093/nb/87291437-1437-5

Rohrer, A. E., Weiss, N., Kokjohn, T. A., Kuo, Y. M., Kalback, W., Anthony, J., Watson, D., Luehrs, D. C., Sue, L., Walker, D., Emmerling, M., Goux, W., & Beach, T. (2002). Increased Aβ peptides and reduced cholesterol and myelin proteins characterize white matter degeneration in Alzheimer's disease. *Biochemistry*, 41(7), 11078–11090. https://doi.org/10.1021/bi02617sd

Saher, G., Brügger, B., Lappe-Siefke, C., Möbius, W., Tozawa, R. I., Wehr, M. C., Wieland, F., Ishibashi, S., & Nave, K. A. (2005). High cholesterol level is essential for myelin membrane growth. *Nature Neuroscience*, 8(4), 468–475. https://doi.org/10.1038/nn1426

Sandrebring-Matton, A., Goikolea, J., Björkhem, I., Patronelain, L., Kemppainen, N., Laikakinen, N., Ngando, T., Rinne, J., Soininen, H., Cedazo-Minguez, A., Solomon, A., & Kivipelto, M. (2021). 27-hydroxycholesterol, cognition, and brain imaging markers in the FINGER randomized controlled trial. *Alzheimer's Research & Therapy*, 13(1), 56. https://doi.org/10.1186/s11139-021-00790-y

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schind, B., Tinevez, J. Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676–682. https://doi.org/10.1038/nmeth.1921

Selhubjerg, F., Börnsten, L., Ammitzbøll, C., Nielsen, J. E., Vinther-Jensen, T., Hjermd, L. E., van Essen, M., Ratzer, R. L., Sørensen, P. S., & Romme Christensen, J. (2017). Defining active progressive multiple sclerosis. *Multiple Sclerosis*, 23(13), 1727–1735. https://doi.org/10.1177/1352458517726592

Snidero, N., Velte, C., Myllykoski, M., Raasakka, A., Ignatev, A., Werner, H. B., Erwig, M. S., Möbius, W., Kursula, P., Nave, K. A., & Simons, M. (2017). Antagonistic functions of MBP and CNP establish cytosolic channels in CNS myelin. *Cell Reports*, 18(2), 314–323. https://doi.org/10.1016/j.celrep.2016.12.053

Staurenghi, E., Cerrato, V., Gamba, P., Testa, G., Giannelli, S., Leoni, V., Caccia, C., Buffo, A., Noble, W., Perez-Nieves, B. G., & Leonardiuzzi, G. (2021). Oxysterols present in Alzheimer's disease brain induce synaptotoxicity by activating astrocytes: A major role for lipocalin-2. *Redox Biology*, 39, 101837. https://doi.org/10.1016/j.redox.2020.101837

Testa, G., Gamba, P., Badilli, U., Gargiulo, S., Maina, M., Guina, T., Calpfapietra, S., Biai, F., Cavalli, R., Poli, G., & Leonardiuzzi, G. (2014). Loading into nanoparticles improves Quercetin's efficacy in preventing neuroinflammation induced by oxysterols. *PLoS One*, 9(5), e96795. https://doi.org/10.1371/journal.pone.0096795

Underhill, H. R., Rostomily, R. C., Mikheev, A. M., Yuan, C., & Yarnyk, V. L. (2011). Fast bound pool fraction imaging of the in vivo rat brain: Association with myelin content and validation in the C6 glioma model. *Neuroimage*, 54(3), 2052–2065. https://doi.org/10.1016/j.neuroimage.2010.10.065

Wingo, T. S., Cutler, D. J., Wingo, A. P., Le, N. A., Rabinovici, G. D., Miller, B. L., Lah, J. J., & Levey, A. I. (2019). Association of early-onset Alzheimer disease with elevated low-density lipoprotein cholesterol levels and rare genetic coding variants of APOB. *JAMA Neurology*, 7(6), 809–817. https://doi.org/10.1001/jamaneurol.2019.0648

Yu, Y., Chen, Y., Kim, B., Wang, H., Zhao, C., He, X., Liu, L., Liu, W., Wu, L. M. N., Mao, M., Chan, J. R., Wu, J., & Lu, Q. R. (2013). Olig2 targets chromatin remodelers to enhance oligodendrocyte differentiation. *Cell*, 152(1–2), 248–261. https://doi.org/10.1016/j.cell.2012.12.006

Zambón, D., Quintana, M., Mata, P., Alonso, R., Benavent, J., Cruz-Sánchez, F., Gich, J., Pocovi, M., Civeira, F., Capurro, S., Bachman, D., Sambamurti, K., Nicholas, J., & Pappolla, M. A. (2010). Higher incidence of mild cognitive impairment in familial hypercholesterolemia. *American Journal of Medicine*, 123(3), 267–274. https://doi.org/10.1016/j.amjmed.2009.08.015

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