Lack of LDL receptor aggravates learning deficits and amyloid deposits in Alzheimer transgenic mice

Dongfeng Cao a, Ken-ichiro Fukuchi b,d, Hongquan Wan a, Helen Kim c, Ling Li a,∗

a Atherosclerosis Research Unit, Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Alabama at Birmingham, 1530 3rd Avenue South, BDB 658, Birmingham, AL 35294, USA
b Department of Genetics, University of Alabama at Birmingham, Birmingham, AL 35294, USA
c Department of Pharmacology, University of Alabama at Birmingham, Birmingham, AL 35294, USA
d Department of Biomedical and Therapeutic Sciences, University of Illinois College of Medicine at Peoria, Peoria, IL 61656, USA

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Abstract
Emerging evidence indicates that cholesterol metabolism affects the pathogenesis of Alzheimer’s disease (AD). The LDL receptor (LDLR) is obligatory in maintaining cholesterol homeostasis in the periphery. To investigate the role of LDLR in the development of AD-like behavior and pathology, Tg2576 mice, a well-characterized transgenic mouse model of AD, with different genotypes of LDLR were generated. Here we show that LDLR-deficient Tg2576 mice developed hypercholesterolemia and age-dependent cerebral \(\beta\)-amyloidosis. Before the manifestation of \(\beta\)-amyloid (\(A\)\(\beta\)) deposition, these mice displayed hyperactivity, reduced anxiety, and impaired spatial learning regardless of LDLR genotypes. After the manifestation of \(A\)\(\beta\) deposition, LDLR-deficient Tg2576 mice showed more spatial learning deficits than LDLR-intact Tg2576 mice. Although LDLR genotypes did not affect the expression level of the amyloid-\(\beta\) precursor protein transgene, there was a significant increase in \(A\)\(\beta\) deposition accompanied with an increase of apoE expression in LDLR-deficient Tg2576 mice. Our results suggest that the LDLR plays a role in the development of Alzheimer-type learning impairment and amyloidosis and can be a novel therapeutic target for AD.

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1. Introduction
Alzheimer’s disease (AD) is a common age-related neurodegenerative disorder characterized clinically by progressive cognitive impairment. Pathological hallmarks of AD brain include neurofibrillary tangles and deposits of aggregated amyloid-\(\beta\) protein (\(A\)\(\beta\)) in neuritic plaques and cerebral vessels [37]. The pathogenic mechanisms that lead to the development of AD, however, are not fully understood. One of the main hypotheses is that \(\beta\)-amyloidosis (production and deposition of \(A\)\(\beta\)) plays a crucial role in the pathogenesis of AD [37]. \(A\)\(\beta\) (39–43 amino acids) is derived from a large transmembrane glycoprotein, amyloid-\(\beta\) precursor protein (APP), by proteolytic processing.

Accumulating evidence indicates that cholesterol is closely involved in the development of AD. Early epidemiological data indicate an increased prevalence of cerebral senile plaques in cognitively intact individuals with heart disease compared to age-matched controls with no heart disease [40], suggesting a possible link between high plasma cholesterol levels and cerebral amyloidosis. The apolipoprotein (apo) E4 allele, a strong risk factor for AD [7], is also associated with hypercholesterolemia and atherosclerosis [7]. Elevated plasma cholesterol levels have been shown to be an independent risk factor for AD [28]. In vitro evidence also links cholesterol with APP processing and amyloid deposition [3,8,9,21,39]. Experimentally, dietary-induced hypercholesterolemia induces the formation of \(A\)\(\beta\) deposition in the brain of rabbits [41]. Recently, we and others have demonstrated that high fat/high cholesterol diets exacerbate cerebral \(\beta\)-amyloidosis in APP transgenic mice [23,24,33,38]. We also have shown that an atherogenic
diet aggravates learning deficits in these mice [24]. Furthermore, recent studies show that acyl-coenzyme A: cholesterol acyltransferase (ACAT) modulates the generation of Aβ in vitro [32] and that an ACAT inhibitor CP-113,818 markedly reduces amyloid pathology in APP transgenic mice [18]. Additionally, in retrospective epidemiological studies, use of cholesterol-lowering statin drugs has been associated with a decreased risk of AD [20,44]. The sum of these data suggests that hypercholesterolemia promotes the development of AD.

Normal cholesterol homeostasis is maintained through interactions between lipoproteins and their receptors. In humans, deficiency in the low-density lipoprotein receptor (LDLR) causes familial hypercholesterolemia (FH) [4]. FH is inherited as an autosomal dominant trait and is the most frequent cause of premature coronary heart disease resulting from a single gene defect [12]. Little is known, however, about the role of LDLR in the central nervous system, although the expression of LDLR is generally believed to be mainly responsible for the pathological changes in RAP-deficient mice. The role of other RAP-dependent receptors including the LDLR, VLDLR, and apoER2, however, could be more important in brain development and influence the pathogenesis of AD [14].

Recent studies suggest that the LDLR is the secondary target for statin treatment. By inhibiting cholesterol biosynthesis and reducing intracellular cholesterol stores, statins upregulate the LDLR and subsequently accelerate the clearance of cholesterol-loaded LDL from plasma [4]. Heterozygous FH is associated with reduced expression of the LDLR [36]. Increased cerebral amyloid deposition and neurodegeneration was observed in APP transgenic mice deficient in receptor-associated protein (RAP) [43]. The LDLR genotype had no effect on the expression level of the APP transgene, LDLR-deficient Tg2576 mice developed significantly increased cerebral amyloid-β deposition and spatial learning deficits compared with age- and sex-matched LDLR-intact Tg2576 mice.

2. Methods

2.1. Animals

Tg2576 mice (APP+ heterozygous), on the C57BL/6XSJL F2 mixed background and overexpressing human APP with the Swedish double mutation [17], were bred with LDLR−/− mice (C57BL/6J, Jackson Laboratory) to produce APP+LDLR+/+ and non-APP transgenic LDLR+/− mice. Further mating of APP+LDLR+/+ mice with LDLR+/− mice produced progenies with six genotypes: APP transgenic (APP+LDLR+/+, APP+LDLR+/−, and APP+LDLR−/−) and non-APP transgenic mice (LDLR+/+, LDLR+/−, and LDLR−/−). The genotypes of these mice were determined by PCR of tail genomic DNA using specific primers. The APP transgene was detected as described previously [24]. The LDLR genotype was determined by the protocol of Jackson Laboratory. Female mice with the following four genotypes, APP+LDLR+/+, APP+LDLR+/−, APP+LDLR−/−, LDLR+/+, LDLR+/−, and LDLR−/−, were used in this study. Mice were housed under standard conditions in conventional cages and given standard rodent diet and water ad libitum. All animal procedures used for this study were prospectively reviewed and approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

2.2. Determination of plasma total cholesterol concentrations and lipoprotein cholesterol profiles

Blood samples were collected from anesthetized animals by retro-orbital bleeding or by cardiac puncture at the end of experiment. Plasma total cholesterol levels were determined colorimetrically by commercial reagents (Infinity™ chores- terol reagent; Thermo Electron Corporation, Melbourne, Australia). Plasma lipoprotein cholesterol profiles were analyzed by the chromatographic method described previously [11].

2.3. Assessment of behavioral functions

Three AD-related behavioral functions, spatial learning and memory, exploration of environmental stimuli, and
anxiety, were assessed. The testing schedule included exploration and motor activity in an open field (days 1–3), an elevated plus-maze (days 4–5), and spatial learning in the Morris water maze (days 6–11). All equipment and software were purchased from SD Instruments Inc., San Diego, CA. All testing procedures were described previously [22,24]. Briefly, the open field is made of white acrylic with a 50 cm × 50 cm surface area and with each wall reaching 38 cm in height. The activity was recorded by an overhead video camera and analyzed by video-tracking SMART software (SD Instruments, San Diego, CA). The mice were placed in the open field for a 5-min session daily for 3 days.

Anxiety was measured in the elevated plus-maze, consisting of four arms in a cross-shaped form and a central region. Two of the arms were enclosed on three sides by walls, whereas the other two were not. The enclosed or open arms of the maze faced each other. The mice were placed in the central region and their behavior recorded for 5 min/session for 2 days. The number of entries and the time spent in either the enclosed or the open arms were measured.

Spatial orientation was evaluated in the Morris water maze consisting of a round basin (diameter: 112 cm) filled with water (22 °C) to a height of 31 cm. The water was made opaque by mixing in dry milk to camouflage the escape platform (8 cm × 8 cm). The pool was placed in a room with abundant extra-maze visual cues. The acquisition of the spatial task consisted of placing the mice next to and facing the wall successively in north (N), east (E), south (S), and west (W) positions, with the escape platform hidden 1 cm beneath water level in the middle of the NE quadrant. In each trial, the mouse was allowed to swim until it found the hidden platform, or until 60 s had elapsed, at which point the mouse was guided to the platform. The mouse was then allowed to sit on the platform for 10 s before being picked up. The escape latency and swim path length (distance) were recorded by the SMART system for four trials daily for 5 days.

The day after the acquisition phase, a probe trial was conducted by removing the platform and placing the mouse next to and facing the N side. The time spent in the NE quadrant was measured in a single 1-min trial. Two hours later, the visible platform version was evaluated, with the escape platform lifted 1 cm above water level and shifted to the SE quadrant. A pole (height = 7 cm) was inserted on top of the escape platform as a viewing aid. In an identical manner to the place learning task, escape latencies and swim path length were measured for four trials, except that the test was conducted in a single day.

2.4. Brain tissue preparation

Mice were anesthetized with sodium pentobarbital, and blood was obtained via cardiac puncture with heparin as an anticoagulant. Following transcardiac perfusion with ice-cold phosphate-buffered saline (PBS) (pH 7.4), brains were divided into left and right hemispheres. The right hemisphere was fixed in phosphate-buffered formalin for subsequent histological analysis. The left hemisphere with the olfactory lobe and cerebellum excised was snap frozen in liquid nitrogen and stored in a −80 °C freezer for subsequent biochemical analysis.

2.5. Immunohistochemical analysis and quantification of cerebral β-amyloidosis

Protocols for immunohistochemical analysis were described previously [24]. Briefly, formalin-fixed and paraffin-embedded tissue sections were subjected to the avidin-biotin immunoperoxidase method to detect the antigens (e.g. Aβ) using Vectastain ABC kit (Vector, Burlingame, CA). Primary antibodies used for assessing β-amyloidosis in the brain of mice included: 6E10 (a monoclonal antibody raised against amino acid 1–16 of Aβ, Signet, Dedham, MA), 4G8 (a monoclonal antibody raised against amino acid 17–24 of Aβ, Signet, Dedham, MA), and a polyclonal rabbit anti-Aβ antibody (raised against a 30 amino acid synthetic peptide derived from full length Aβ; Zymed, San Francisco, CA). The amyloid load in the cortex and hippocampus of mouse brain were quantified by the histomorphometry system consisting of a Leica DMR research microscope equipped for fluorescence, polarizer analyzer, and brightfield microscopy, a SPOT RT Slider digital camera (Diagnostic Instruments, Sterling Heights, MI), and the Image Pro Plus v4 image analysis software (Media Cybernetics, Silver Spring, MD) capable of color segmentation and automation via programmable macros. Multiple images of 1 mm² each were captured and analyzed from 5 coronal brain sections at 500-μm intervals from each mouse using a 10× objective lens. A total area of 50 mm² giving the highest total Aβ immunoreactivity was chosen to calculate the amyloid load expressed as a percentage of total area covered by Aβ immunoreactivity.

2.6. Brain cholesterol and immunoblot analysis

Frozen brain samples were Dounce homogenized in carbonate buffer (100 mM Na₂CO₃, 50 mM NaCl, pH 11.5) containing protease inhibitors (10 μg/ml aprotinin and 1 mM 4-(2-aminoethyl)benzenesulfonic fluoride hydrochloride (AEBSF)). An aliquot of homogenate was subjected to further homogenization with guanidine hydrochloride (final concentration, 5M). The homogenate was rock-shaken for 3–4 h at the room temperature and diluted for cholesterol analysis by the Infinity™ cholesterol reagent (Thermo Electron Corporation, Melbourne, Australia). Another aliquot of carbonate homogenate was subjected to further homogenization with the SDS sample buffer (In vitrogen, Caesbad, CA), boiled for 5 min, and sheared with 26-gauge needles. Protein concentrations were determined by the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA). Fifty micrograms of proteins for each sample were separated by SDS-PAGE, and blotted to PVDF membranes. The membranes were incubated with primary antibodies.
followed by biotinylated secondary antibodies. Signal was detected by the Western Lightning Chemiluminescence Reagent Plus (Perkin-Elmer Life Sciences, Boston, MA) and quantified by densitometric scanning using the LabWorks image acquisition and analysis software (UVP Inc., Upland, CA). Primary antibodies used for immunoblot analysis included: goat polyclonal antibody against mouse apoE (Santa Cruz Biotechnology, Santa Cruz, CA), rabbit polyclonal antibody 994B against the 39 carboxyl-terminal residues of human APP [10], and monoclonal antibody against actin (Chemicon International, Temecula, CA).

2.7. Statistical analysis

Data were expressed as mean ± standard error. Comparison of different genotype groups was performed by two-tailed Student’s t-test (for normally distributed data), Mann–Whitney rank sum test (for non-normally distributed data) and repeated measures analysis of variance (ANOVA). In probe tests, each group was compared by Mann–Whitney rank sum test to a theoretical group performing at chance, defined as 25% for the four-choice probe trial. Correlations were determined by Pearson product moment correlation analysis. The SigmaStat software (SPSS Science, Chicago, IL) was used for statistical analyses. P < 0.05 was considered statistically significant.

3. Results

3.1. Generation of LDLR-deficient Tg2576 mice

Tg2576 mice with different genotypes of LDLR were generated by two steps crossbreeding as described in Section 2. The total numbers of mice produced for each genotype (APP+LDLR+/+, APP+LDLR+/−, APP+LDLR−/−, LDLR+/+, LDLR+/−, and LDLR−/−) were 34, 84, 28, 42, 95, and 59, respectively, at weaning. The ratio did not differ from the expected 1:2:1:1:2:1 ratio analyzed by the χ2-test (P = 0.206). All mice appeared healthy and there were no apparent differences in body weight, physical appearance, behavior in cage, or handling among groups.

Plasma cholesterol levels of mice were measured at 10 months of age. As expected, APP+LDLR−/− and LDLR−/− mice showed hypercholesterolemia with plasma cholesterol levels similar to heterozygous FH humans (Fig. 1A). The increase of cholesterol occurred in the fraction of non-high-density lipoproteins (non-HDL) as the HDL cholesterol levels did not differ among the four groups (Fig. 1A). Plasma lipoprotein cholesterol profile analyses showed that cholesterol levels were increased mainly in the fraction of LDL in the APP+LDLR−/− and LDLR−/− mice (Fig. 1B). Furthermore, total plasma cholesterol levels and lipoprotein cholesterol profiles were determined only by the genotype of LDLR and were not affected by the presence or the absence of the APP transgene (Fig. 1A and B).

Brains from APP+LDLR−/− mice at different ages were examined for Aβ deposition by immunohistochemistry. APP+LDLR−/− mice developed age-dependent cerebral amyloidosis (Fig. 2). Amyloid plaques were first seen in the hippocampus and cortex of mice at 11 months of age and accumulated progressively afterwards (Fig. 2).

3.2. Exploration of the environment stimuli and anxiety

The exploration of environmental stimuli and anxiety were assessed because lack of initiative and decreased anxiety levels are common neuropsychiatric features of AD [6]. The exploration of environmental stimuli was assessed in an open-field test. The elevated plus-maze was used as an anxiety test where mice choose to explore either anxiogenic (open) or safer (enclosed) arms [29]. These tests were conducted with mice at 10 months of age.

In the open-field test, two parameters, the path length (distance traveled) and resting time (as a percentage of total testing time), were used to assess exploratory activities of different groups of mice. Although APP+LDLR+/+ mice...
Fig. 2. Age-dependent cerebral Aβ deposition in APP+LDLR−/− mice. Brain sections from mice at the age of 11 (A), 13 (B), 15 (C), and 24 (D) months were immunostained with anti-Aβ antibody 6E10 and counterstained with hematoxylin. Three mice at each age (except only one mouse at 24 months old) were examined. Pictures shown represent an average cerebral Aβ deposition for each age group (bar = 200 μm).

Fig. 3. Activity measurements in the light-dark box. (A) Comparison of path lengths between APP+LDLR−/− and APP+LDLR+/+ mice during the 3-day testing as a whole. The activity of the APP+LDLR+/+ mice, however, decreased significantly on the 2nd day (P < 0.05), as a result of intersession habituation. The APP+LDLR−/− mice, on the other hand, showed no intersession habituation over 3 days of testing. The slope of the activity curve was essentially flat. In the non-APP transgenic mice, there were also no significant differences in activities between LDLR−/− and LDLR+/+ mice (F(1,120) = 0.25, P = 0.628). In these mice, however, the slope of the activity curve declined sharply during the 3 days of testing (F(2,28) = 45.98, P < 0.001) independent of LDLR genotypes (Fig. 3B).

3.3. Spatial learning and memory

As anterograde amnesia is the earliest feature of AD [31], the assessment of spatial learning and memory is central to AD. The spatial learning and memory ability of the mice were measured in the Morris water maze test [25]. To determine the effect of amyloid deposition on spatial learning and memory, mice were assessed at the ages before (10 months) and after (13 months) the manifestation of cerebral amyloidosis.

At 10 months of age, both APP+LDLR−/− and APP+LDLR+/+ mice learned the location of the submerged platform with repeated testing (F(1,59) = 15.91, P < 0.001), shown by the decreasing escape latency (Fig. 5A) and path length (Fig. 5D) over the 5 testing days in the acquisition phase of the test. For non-APP transgenic mice, the performance was also independent of the LDLR genotype.

LDLR−/− mice performed similarly as LDLR+/+ mice (Fig. 5B and E). When the data from APP+ and non-APP transgenic mice were pooled, regardless of LDLR genotypes, APP+ mice demonstrated spatial learning impairment as compared to non-APP transgenic mice as shown by increased...
escape latency (Fig. 5C, $F_{(1,12)} = 11.05, P = 0.002$) and path length (Fig. 5F, $F_{(1,12)} = 38.24, P < 0.001$).

In the 60 s probe trial, no significant differences were observed among the groups. All groups appeared to spend more time than chance (25%) in the target quadrant. The percent time spent in the target quadrant was 34.6 ± 3.1, 35.7 ± 5.6, 37.6 ± 6.5, and 33.4 ± 6.6% for APP+LDLR−/−, APP+LDLR+/+, LDLR−/−, and LDLR+/+ mice, respectively. These results indicated that these mice had similar retention of spatial memory at this age. In the visible platform version of the Morris water maze test, all groups performed similarly. The escape latencies for the four genotype groups above were 4.5–16.7 s, 9.8 ± 1.8 s (range 5.4–18.1 s), 10.9 ± 1.3 s (range 5.4–16.1 s), and 8.2 ± 1.0 s (range 4.4–17.2 s), respectively. These results indicate that visual acuity and swimming abilities did not differ among the groups.

Three months later at 13 months of age, these same animals were again subjected to the paradigm of the Morris water maze test. Significant differences were observed in the performance of APP+LDLR−/− and APP+LDLR+/+ mice. APP+LDLR+/+ mice re-acquired the location of the hidden platform significantly faster than APP+LDLR−/− shown by shorter escape latency (Fig. 6A, $F_{(1,56)} = 6.48, P = 0.023$) and path length (Fig. 6C, $F_{(1,56)} = 4.93, P = 0.043$). There were still no differences, however, in the acquisition of the
3.4. Increased cerebral β-amyloid deposition in LDLR-deficient Tg2576 mice

To determine whether the LDLR genotype affects the extent of cerebral amyloidosis, brain sections from 13-month-old APP+LDLR+/− (n = 11) and APP+LDLR+/+ (n = 10) littermates were subjected to immunohistochemical and morphometrical analyses for quantification of Aβ load. APP+LDLR+/− mice had an about 2-fold increase in cerebral amyloid load (P = 0.028) compared with APP+LDLR+/+ mice (Fig. 7A), although a greater variation of cerebral amyloidosis was observed in APP+LDLR+/− mice. To confirm the difference was not attributed to any outliers, the data were further analyzed by the PROC MULTTEST procedure in SAS that was specifically designed to allow for non-normality using bootstrap and permutation re-sampling methods. This procedure generates bootstrap data by re-sampling with replacement from the pooled residuals. Analyses with this procedure resulted in a P-value of 0.026 that agreed well with the P-value (0.028) obtained above using the normality assumption. To determine how the cerebral amyloid load affects the performance of the mice in the Morris water maze test, the escape latencies on day 2 were plotted as a function of corresponding cerebral amyloid load (Fig. 7B).

The results showed that there was a significant positive correlation between the two measurements (Pearson correlation r = 0.51, P = 0.021).

To determine if the LDLR genotype affects the expression of the human APP transgene, cerebral homogenates from APP+LDLR+/+ and APP+LDLR+/− mice were subjected to immunoblot analysis. No significant differences were observed in the steady state APP levels between the two groups (Fig. 8A and B). Since apoE plays a vital role in β-amyloidosis, the expression levels of apoE were also determined. The levels of apoE in the brain of APP+LDLR+/− mice increased about 2-fold compared with those in APP+LDLR+/+ mice (Fig. 8A and B). Cerebral total cholesterol content, however, was not affected by the LDLR genotype (18.0 ± 0.4 (n = 9) versus 17.5 ± 0.5 (n = 8) ng/mg wet weight, APP+LDLR+/+ versus APP+LDLR+/−, P = 0.42).

4. Discussion

In the present study, we successfully generated APP+LDLR+/− mice that develop FH-like hypercholesterolemia, age-dependent cerebral amyloidosis, and behavioral deficits. Our results showed that, in the brain of APP+LDLR+/− mice, Aβ deposition occurred at about 11 months of age and accumulated progressively during aging. Before the manifestation of cerebral Aβ deposition at the age of 10 months, neurobehavioral functions did not differ between APP+LDLR+/− and APP+LDLR+/+ mice. Both groups displayed hyperactivity, reduced anxiety, and impaired spatial learning compared with non-APP...
transgenic littermates at this age. After the manifestation of Aβ deposition in the brain by 13 months of age, however, APP+LDLR−/− mice showed increased impairment of spatial learning compared with APP+LDLR+/+ mice. Consistent with this exacerbation of learning impairment, APP+LDLR−/− mice had significantly more cerebral Aβ deposition than APP+LDLR+/+ mice. To our knowledge, this study is the first to show that the LDLR may play a direct role in the development of AD-type neuropathology.

Increased cerebral Aβ deposition in APP+LDLR−/− mice may be caused by either increased production, aggregation, or decreased clearance of Aβ. The LDLR genotype did not appear to affect the age of onset for Aβ deposition. The expression levels of the human APP transgene did not differ between APP+LDLR−/− and APP+LDLR+/+ mice. Preliminary studies showed that the total Aβ levels in the brain of APP+LDLR−/− mice increased about 2-fold compared with those of APP+LDLR+/+ mice and that the increase was found only in the fraction of insoluble Aβ and not in the fraction of soluble Aβ (Cao and Li, unpublished data). These results suggest that the amyloidogenic effect of LDLR deficiency resulted from decreased Aβ clearance and increased Aβ aggregation rather than from increased expression of the human APP transgene or increased Aβ production.

The role of LRP in Aβ clearance has been well established [42], whereas the role of LDLR has not been directly investigated. Van Uden et al. reported that LRP appeared to be the main lipoprotein receptor responsible for Aβ clearance but could not exclude the possibility that LDLR might also be involved in Aβ clearance [43]. Results from the present study strongly suggest the LDLR as an additional receptor mediating Aβ clearance. In this regard, upregulation of the LDLR by statins may provide another explanation for reduced Aβ levels observed in statin-treated animals [8,30]. Furthermore,
cerebral apoE levels were increased in APP+LDLR−/− mice. ApoE, an LDLR ligand, binds Aβ and enhances Aβ deposition [1,2]. Lack of apoE leads to a marked decrease of Aβ deposition in the brain of APP transgenic mice [1,2], whereas expression of apoE facilitates the formation of cerebral amyloid plaques [15,16]. As discussed above, a 2-fold increase of insoluble Aβ levels were observed in the brain of APP+LDLR−/− mice (Cao and Li, unpublished data). These results support the concept that apoE contributes to increased cerebral Aβ aggregation and deposition in APP+LDLR−/− mice.

A confounding factor in interpreting the results from LDLR-deficient Tg2576 mice is the hypercholesterolemia that accompanies LDLR-deficiency. It is possible that both the LDLR deficiency per se and the resulting hypercholesterolemia contribute to the increased cerebral Aβ deposition in APP+LDLR−/− mice. Mean cerebral total cholesterol content in APP+LDLR−/− mice, however, did not differ from that of APP+LDLR+/+ mice, indicating that the absence of the LDLR did not appear to affect the brain cholesterol homeostasis. Further studies are needed to dissect the effect of LDLR deficiency from that of hypercholesterolemia. A study is underway in our laboratory in which APP+LDLR−/− mice are treated with a cholesterol-lowering drug, ezetimibe (Zetia®, Merck.Schering-Plough Pharmaceuticals, Kenilworth, NJ). Ezetimibe inhibits the absorption of biliary and dietary cholesterol from the small intestine [19] and effectively lowers plasma cholesterol levels in LDLR−/− mice [34]. Alternatively, it is possible to generate animal models of AD with tissue specific knockout of the LDLR in the brain to exclude the effect of hypercholesterolemia. APP+LDLR−/− mice showed a greater variation of cerebral Aβ deposition than APP+LDLR+/+ mice (data not shown). These results suggest that hypercholesterolemic status may not be a major factor determining the extent of cerebral Aβ-amyloidosis in these mice. A more likely explanation is that the LDLR deficiency exerts differential effects on Aβ deposition in mice with different genetic makeup as these mice are in a mixed (C57BL/6 and SJL) genetic background. The importance of the LDLR deficiency in the clearance of Aβ may vary depending on its interactions with certain genetic factors, resulting in a larger variation of cerebral Aβ-amyloidosis in genetically heterogeneous animals. As human populations are genetically heterogeneous, this speculation may also be true in humans. The presence or absence of the LDLR did not affect the behavioral functions tested in non-APP transgenic mice.
Fig. 7. (A) Cerebral Aβ load. Brain sections from APP+LDLR−/− (n = 11) and APP+LDLR+/+ (n = 10) mice at 13 months of age were subjected to immunohistochemical and morphometrical analyses for quantification of Aβ load as described in Section 2. APP+LDLR−/− mice developed significantly more cerebral Aβ deposition than APP+LDLR+/+ mice. *P < 0.05. (B) Correlation of escape latencies with cerebral amyloid load of the mice. Pearson correlation r = 0.51, P = 0.021.

These results differ from a previous report by Mulder et al. [26] who showed that LDLR−/− mice display impaired spatial memory retention in the probe trial, although they also did not observe any differences between LDLR−/− and LDLR+/+ mice in spatial learning and memory performance in the acquisition phase of the water maze [26]. The discrepancy could be caused by differences in the sex and genetic background of animals between the two studies. In the study of Mulder et al. [26], all mice were male and on the C57BL/6 background, whereas in the present study, all mice were female and in a mixed C57BL/6 SJL background. Future studies including both sexes in both genetic backgrounds should be able to address this issue.

Fig. 8. Cerebral expression levels of APP and apoE. Cerebral homogenates from APP+LDLR−/− (n = 4) and APP+LDLR+/+ (n = 4) mice at 13 months of age were subjected to immunoblot analysis as described in Section 2. (A) Images of immunoblots with antibodies against APP, apoE, and actin, respectively. (B) Densitometric analysis of APP and apoE blots in (A) with the expression levels in APP+LDLR+/+ set as 100%. Expression levels of APP were not different between APP+LDLR−/− and APP+LDLR+/+ mice. Expression levels of apoE were increased about 2-fold in APP+LDLR−/− mice. Actin blot was used for loading control showing no loading differences among samples (P > 0.05).

In APP transgenic mice, however, APP+LDLR−/− displayed increased impairment in spatial learning as compared to APP+LDLR+/+ mice after the manifestation of cerebral amyloidosis. This aggravation of learning impairment most likely resulted from the effect of LDLR deficiency on cerebral Aβ deposition in APP+LDLR−/− mice rather than the LDLR deficiency per se because non-APP transgenic LDLR−/− littermates performed normally. Indeed among cognitively intact humans (67 years of age), FH patients perform similarly to non-FH individuals in a learning test [13]. The authors suggested that FH-causing mutations might have effects on learning and memory among people over the age for AD. Results from the present study with APP+LDLR−/− mice support this possibility. It will be interesting to investigate if AD patients with FH develop more learning and memory deficits. More than 600 mutations causing FH have been identified [27]. To our knowledge, however, there has been no report on the association of FH-causing LDLR mutations
with AD. This may be explained by the fact that FH patients often die before the age of 70, which is a relatively young age for the manifestation of AD. Interestingly, two polymorphisms in exons 8 and 13 of the LDLR have been associated with AD [35]. Cheng et al. recently show that the LDLR isoforms with these two polymorphisms may interact with apoE4 in determining AD risk [AD5]. In addition, another recent report shows an association of a specific LDLR haplotype with AD [13]. These findings warrant further studies on the role of LDLR in the development of AD.

In summary, the present study showed that APP+/
LDLR−/− mice develop increased cerebral Aβ deposition and spatial learning impairment, supporting that the LDLR plays a role in the pathogenesis of AD. These results suggest that the LDLR may be a novel target for developing therapeutic strategies for AD.

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