IMPACT OF HUMAN APP OVEREXPRESSION ON CEREBRAL CHOLESTEROL METABOLISM IN MICE

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BACKGROUND

Processing of APP and Abeta has been in the center of Alzheimer's disease (AD) research for decades. Beside many other variables, lipids, especially cholesterol and its derivatives, are discussed to contribute to AD pathogenesis. Several studies show that cholesterol affects APP metabolism. Interestingly, also the converse mechanism, the direct influence of Aβ on cholesterol metabolism, has been described. The presented study investigates whether human APP overexpression and changes in Aβ generation influence cholesterol metabolism in $hAPP_{SL}$ mice, a well-established model of brain amyloidosis and AD.

MATERIALS AND METHODS

Changes in cholesterol content in cortical and residual brain samples of 6 months old $hAPP_{SL}$ and WT animals were measured after Folch-extraction with BioVision Cholesterol Quantitation Kit. mRNA levels of cholesterol metabolism-associated genes were measured in brain tissue of 6 months old $hAPP_{SL}$ mice and non-transgenic littermates with SYBR Green quantitative mRNA RT-PCR. To additionally investigate the impact of dietary cholesterol on these parameters, brain tissue of $hAPP_{SL}$ and wild type (WT) mice that received a high-fat/high-cholesterol diet (HFD) for three months was examined for mRNA expression as well .

RESULTS

In 6 months old animals free cholesterol and cholesteryl esters were quantified using cortical and remaining brain tissue. A shift towards decreased free and increased esterified cholesterol levels was observed in cortical but not in residual brain samples of $hAPP_{SL}$ mice (Fig. 1). Free cholesterol levels in cortices of $hAPP_{SL}$ mice were reduced more than 50% compared to WT littermates (Fig. 1B) .

Higher levels of free cholesterol in the remaining brain compared to the cortex are expected since the remaining brain mainly consists of white matter, which contains high amounts of cholesterol in the myelin sheaths (Fig. 1A,B).

Only small amounts of cholesteryl esters were detected in WT cortices, with levels below 1 µg/mg tissue, but increased to more than 3 µg/mg tissue in $hAPP_{SL}$ animals (Fig 1D). In the remaining brain, no difference in cholesteryl ester levels between the genotypes was observed (Fig. 1C).

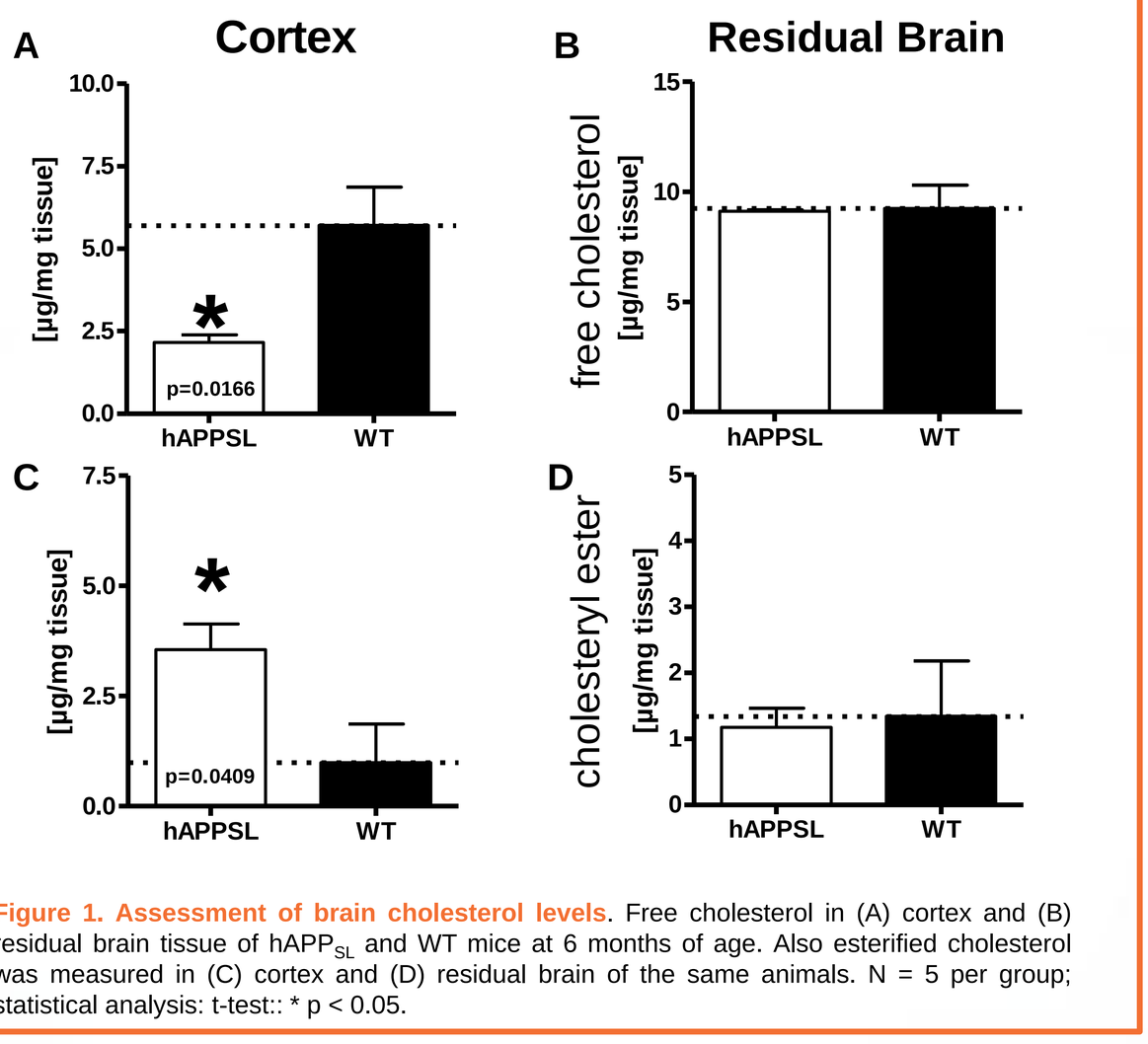

Figure 1. Assessment of brain cholesterol levels. Free cholesterol in (A) cortex and (B) residual brain tissue of $hAPP_{SL}$ and WT mice at 6 months of age. Also esterified cholesterol was measured in (C) cortex and (D) residual brain of the same animals. N = 5 per group; statistical analysis: t-test:: * $p < 0.05$.

RESULTS

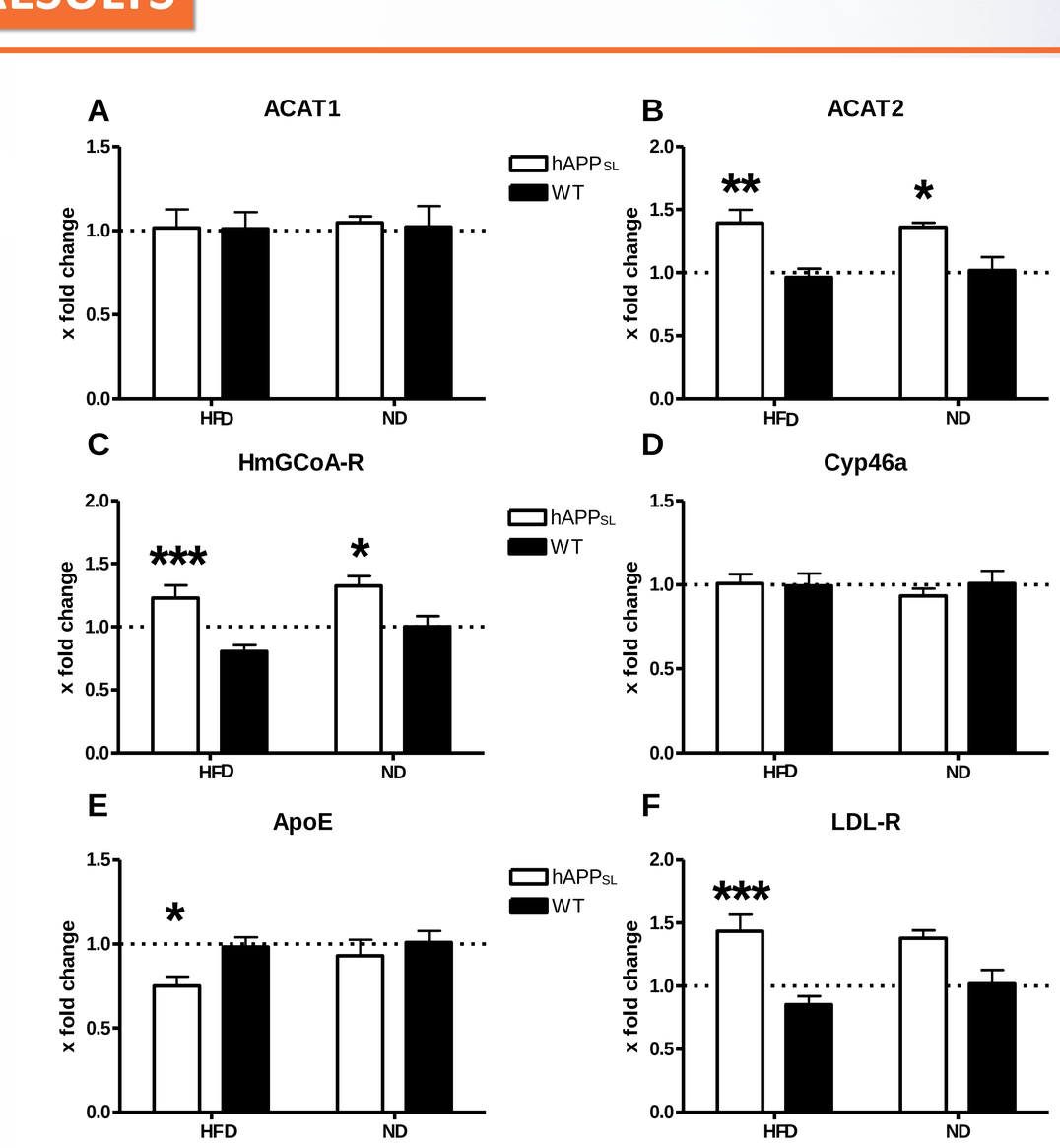

Figure 2. Influence of $hAPP_{SL}$ overexpression and diet on mRNA levels of cholesterol metabolism-associated genes. mRNA expression levels in the cortex of 6 months old $hAPP_{SL}$ mice shown as x-fold change to levels in corresponding WT animals on standard diet (ND) or high fat diet (HFD). (A) Acetyl-CoA−Acetyltransferase 1 , (B) Acetyl-CoA−Acetyltransferase 2 , (C) HMG-CoA-Reductase , (D) Cholesterol 24-hydroxylase , (E) Apolipoprotein E and (F) LDL-Receptor mRNA levels; n = 6 per group. Statistical analyses: Two-way-ANOVA with Bonferroni post-test compared to WT: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

To study a potential crosstalk between APP and cholesterol pathways, mRNA expression of several genes related to cholesterol metabolism and cholesterol transport was analyzed in cortical samples.

Since ACAT2 mRNA levels are increased in $hAPP_{SL}$ mice brains (Fig. 2B), it is feasible that $hAPP_{SL}$ overexpression and/or excess levels of a proteolytic product of APP is causative of this effect. An increase in ACAT may enhance a decrease in free cholesterol as a consequence of cholesteryl ester (CE) production. Notably, ACAT1 mRNA levels were not influenced by genotype or diet (Fig. 2A). Also mRNA levels of another enzyme regulating cerebral cholesterol levels, Cyp46a, by converting free cholesterol to 24S-hydroxycholesterol, was not affected by genotype or diet (Fig. 2D).

On the other hand, a higher expression level of HMGCoA-R mRNA was observed in $hAPP_{SL}$ mice, indicating a possible adaptive mechanism to maintain the supply of cholesterol by boosting HMGCoA-R expression (Fig. 2C). In line, high levels of LDL-R mRNA were found in the cortices of HFD-fed $hAPP_{SL}$ mice compared to WT littermates (Fig. 2F), indicative for the low free cholesterol level of the respective cells.

Interestingly, mRNA levels of ApoE, a main cholesterol transporter in the CNS, but also responsible for Aβ clearance, was downregulated in $hAPP_{SL}$ mice on HFD compared to WT littermates (Fig. 2E).

Grimm et al. (2005) showed that cells lacking APP or a functional γ-secretase exhibit an increase in cellular cholesterol levels, which can be reversed by treatment with Aβ1-40. Therefore, a regulatory feedback loop between Aβ and cholesterol was proposed, indicating APP as cellular sensor for increased cholesterol levels, leading to higher Aβ formation which in turn down regulates cholesterol synthesis.

Results of the present study strongly support this hypothesis from a different point of view, by showing a significant decrease of free cholesterol in brains of mice "chronically exposed" to APP overexpression and therefore high Aβ levels.

GRIMM, M. et al. 2005. Regulation of cholesterol and sphingomyelin metabolism by amyloid-beta and presenilin. *Nat Cell Biol, 7, 1118-23.*

CONCLUSIONS

Changes of cortical cholesterol levels and mRNA expression patterns under normal diet and HFD conditions argue for an important role of APP in cerebral lipid metabolism, pointing towards a possible connection between APP overexpression and the amount and distribution of cerebral cholesterol.

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