RESEARCH PAPER

Apolipoprotein E genotype affects tissue metallothionein levels: studies in targeted gene replacement mice

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Abstract The apolipoprotein E (APOE) genotype is an important risk factor for ageing and age-related diseases. The APOE4 genotype (in contrast to APOE3) has been shown to be associated with oxidative stress and chronic inflammation. Metallothioneins (MT) exhibit antioxidant and anti-inflammatory activity, and MT overexpression has been shown to increase lifespan in mice. Interactions between APOE and MT, however, are largely unknown. Hence, we determined the effect of the APOE4 versus APOE3 genotype on MT levels in targeted gene replacement mice. APOE4 versus APOE3 mice exhibited significantly lower hepatic MT1 and MT2 mRNA as well as lower MT protein levels. The decrease in hepatic MT protein levels in APOE4 as compared to APOE3 mice was accompanied by lower nuclear Nrf1, a protein partly controlling MT gene expression. Cell culture experiments using hepatocytes identified allyl-isothiocyanate (AITC) as a potent MT inductor in vitro. Therefore, we supplemented APOE3 and APOE4 mice with AITC. However, AITC (15 mg/kg b.w.) could only partly correct for decreased MT1 and MT2 gene expression in APOE4 mice in vivo. Furthermore, cholesterol significantly decreased both Nrf1 and MT mRNA levels in Huh7 cells indicating that differences in MT gene expression between the two genotypes could be related to differences in hepatic cholesterol concentrations. Overall, present data suggest that the APOE genotype is an important determinant of tissue MT levels in mice and that MT gene expression may be impaired by the APOE4 genotype.

Keywords APOE · Metallothionein · Nrf1 · Ageing · Mice

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Introduction

Apolipoprotein E (APOE) is a 34-kDa protein, containing 299 amino acids, which is mainly synthesized by the liver but also in brain and macrophages (Newman et al. 1985; Williams et al. 1985; Basu et al. 1981). The APOE gene is polymorphic with three common alleles, coding for three major APOE isoforms designated APOE2, APOE3 and APOE4. The APOE isoforms differ in the amino acids at positions 112 and 158 of the mature protein (Weisgraber et al. 1981). APOE2 has two cysteines, APOE3 has Cys-112 and Arg-158, and APOE4 has two arginines in these positions. The three alleles differ in their frequencies: APOE3 represents 65–70%, while APOE4 carriers account for up to 15–20% of the Caucasian population (Mahley et al. 2009). Presence of the APOE4 allele is associated with a significantly higher risk of age-related chronic diseases including coronary heart disease (CHD) (Humphries et al. 2001; Stephens et al. 2008) and Alzheimer's disease (AD) (Corder et al. 1993). Cohort studies revealed that the E4 allele of APOE was significantly less frequent in centenarians than in younger controls (Schachter et al. 1994). There is increasing evidence demonstrating that APOE4 may be associated with elevated oxidative stress (Dietrich et al. 2005) and chronic inflammation (Jofre-Monseny et al. 2007), which may in turn contribute to the increased CHD and AD risk in this subgroup of the population.

Metallothioneins (MT) are low molecular weight cysteine-rich intracellular proteins with a high binding affinity for essential transition metals, such as zinc and copper. Metallothioneins exist in different isoforms characterized by the length of amino acid chain (MT1–MT4), but MT1 and MT2 are the most widely distributed MT isoforms (Swindell 2011). MT1 and MT2 are expressed in numerous tissues including liver, kidney, heart and lung. MT gene transcription is rapidly induced by transition and heavy metals as well as by oxidative stress (Dalton et al. 1994; Palmiter 1987). MT exhibit antioxidant properties (Miles et al. 2000) and may play a role in the prevention of atherosclerosis (Giacconi et al. 2008). MT might prevent high fat diet-induced cardiac dysfunction (Dong et al. 2007), and overexpression of MT in the heart has been demonstrated to increase lifespan in mice (Yang et al. 2006). Furthermore, overexpression of metal responsive transcription factor 1 (Mtf1) ameliorated lifespan reductions associated with oxidative stress in *Drosophila melanogaster* (Bahadorani et al. 2010). Nevertheless, the functional role of MT in the ageing process still remains to be elucidated (Mocchegiani et al. 2011).

The MT promoter comprises several response elements that regulate its transcription, including the metal response elements (MRE), which are activated by Mtf1 in the presence of dietary zinc and copper (Radtke et al. 1993; Bittel et al. 1998). Furthermore, the antioxidant response element (ARE) mediates MT expression in response to reactive oxygen species and may act synergistically with MRE (Dalton et al. 1994; Andrews 2000). The nuclear factors erythroid-derived 2-related factor 1 (Nrf1) and factor 2 (Nrf2), both members of the Cap 'n' collar (CNC) subfamily of basic leucine zipper (ZIP) transcription factors, bind to ARE (Biswas and Chan 2010; Venugopal and Jaiswal 1996; Brigelius-Flohe and Flohe 2011). Recent data indicate that the expression of MT1 and MT2 genes is largely dependent on Nrf1 (Ohtsuji et al. 2008). Both Nrf1 and Nrf2 bind to ARE, but only Nrf1 may form a complex that activates transcription of the MT1 gene. Little is known about the interaction between APOE and MT, both proteins centrally involved in the ageing process. Therefore, we investigated the effect of the APOE genotype on MT levels in APOE3 and APOE4 targeted gene replacement mice.

Allyl-isothiocyanate (AITC) belongs to the family of isothiocyanates and occurs in many cruciferous vegetables such as cauliflower, Brussels sprouts, cabbage and kale (Kushad et al. 1999; Rungapamestry et al. 2006). AITC is derived from its precursor sinigrin (a glucosinolate) following myrosinase-catalysed hydrolysis (Krul et al. 2002; Bhattacharya et al. 2010). AITC directly interacts with sulfhydryl groups (Zhang et al. 2005) and predominantly conjugates with cysteine residues (Zhang et al. 2010).

AITC has been shown to induce several phase II and antioxidant enzymes, including GST, HO-1 and NQO1 (Zhang and Talalay 1998; Ye and Zhang 2001; Tang and Zhang 2004; Jeong et al. 2005; Bogaards et al. 1990; McWalter et al. 2004). Since AITC has been suggested to be less cytotoxic than sulforaphane (Wagner et al. 2011), another isothiocyanate that has been recently reported to induce MT in human hepatocytes (Hu et al. 2004; Yeh and Yen 2005), we investigated whether dietary AITC may induce MT levels in Huh7 cells and could correct for decreased hepatic MT and Nrf1 mRNA levels in APOE4 mice.

Materials and methods

Mice and diets

Female homozygous APOE3 and APOE4 targeted replacement mice (6–8 weeks old) 'humanized' for the APOE gene were purchased from Taconic Europe (Ry, Denmark). In order to verify respective APOE genotypes of the humanized targeted replacement mice, the two single-nucleotide polymorphisms in the APOE gene (rs429358, rs7412) were determined using TaqMan® SNP genotyping assays by Applied Biosystems according to manufacturer's instructions (Carlsbad CA, USA). In brief, $40\times$ TaqMan® SNP genotyping assay was diluted 1:2 in TE buffer. Subsequently, the $20\times$ TaqMan® SNP genotyping assay was mixed with $2\times$ TaqMan® Universal Mastermix, bidest. water and DNA to a $1\times$ dilution and a final reaction volume of 10 μl. For each reaction, 20 ng DNA was implemented. Control samples with known APOE genotypes were included. PCR was performed using a Rotor Gene 3000 thermal cycler (Corbett research). Liver APOE levels of the two isoproteins were not different between the two genotypes. Mice were kept in macrolon cages (3–4 animals per cage) at 21–25°C with a 12-h day–night cycle and according to the German Regulations for Animal Welfare approved by the local authority (MLUR, Kiel, Germany, No. V312-72241.121-33 (88-7/09)). Semi-synthetic diets ('Western-type diets'), based on corn starch (14.5%), casein (17.1%), sucrose (32.8%) and butter fat (21.2%), containing 48 mg iron, 39 mg zinc and 11 mg copper per kg diet were purchased from Ssniff Special Diets (Soest, Germany). Diets and water were provided ad libitum. Study 1 was conducted using eight APOE3 and eight APOE4 mice per group, respectively. Study 1 lasted 6 weeks. Samples of study 1 were used for MT, Nrf1, Mtf1 as well as zinc, copper and iron analysis in response to the APOE genotype. Study 2 was carried out with a two-factorial experimental design. One group of each APOE genotype was supplemented with either 15 mg AITC/kg

body weight (Sigma-Aldrich, Steinheim, Germany) or solvent (PBS) by oral gavage for 7 days. Study 2 was conducted to determine whether dietary AITC may correct for decreased hepatic MT and Nrf1 mRNA levels in APOE4 mice. The experimental designs of mouse study 1 and 2 are summarized in Table 1.

At the end of the experimental trial, mice were fasted for 12 h prior anaesthesia with carbon dioxide and killed by decapitation. In both mouse studies, food intake (recorded daily) and final body weights (recorded weekly) were not significantly different between groups.

Tissue preparation

Liver, heart, kidney and lung were excised and dissected, one part was stored in RNAlater™ (Qiagen, Hilden, Germany), and the remainder was snap-frozen in liquid nitrogen and stored at −80°C prior usage.

Cell culture

Human hepatoma cells (Huh7; Institute of Applied Cell Culture, Munich, Germany) were maintained in Dulbecco's modified Eagle's medium containing 4.5 g/l glucose, 4 mmol/l L-glutamine, 1 mmol/l sodium pyruvate, 10% foetal calf serum (FCS), 100 U/ml penicillin and 100 µg/ml streptomycin (all PAA, Coelbe, Germany). Cells were grown in 5% CO_2 at 37°C under a humidified atmosphere. AITC was dissolved in DMSO, and cholesterol was dissolved in ethanol. Final solvent concentrations in cell culture medium were <0.1%.

RNA isolation and real-time PCR

Huh7 cells were seeded at an initial density of 250,000 cells/well in 12-well plates and incubated with medium supplemented with either AITC (10, 25 µmol/l) or 20 mg/ml cholesterol for 6 h. RNA from Huh7 cells as well as from liver, heart, kidney and lung of APOE3 and APOE4 transgenic mice (20–30 mg) was isolated with TRIsure following the manufacturer's instructions (Bioline, Luckenwalde, Germany) and quantified photometrically (Spectrophotometer DU800; Beckman Coulter, Krefeld, Germany). Primers for murine and human MT1, MT2, Nrf1, GAPDH and β-actin (Table 2) were designed by standard tools (Spidey, Primer3, NCBI Blast) and purchased from MWG (Ebersberg, Germany). Quantitative real-time PCR was performed as one-step procedure using Sensi-Mix™ one-step kit (Quantace, Berlin, Germany) with SybrGreen detection using the Rotorgene 6000 cycler (Corbett Life Science, Sydney, Australia). Quantitation was done by the use of a standard curve. Transcription levels of target genes were related to transcription of the housekeeping gene GAPDH or β-actin.

Western blot analysis

Liver nuclear extracts for Western blotting were prepared as described in Wagner et al. (Wagner et al. 2010). Membranes were probed with antibodies against Nrf1 and Mtf1 (1:500; Santa Cruz Biotechnology, Heidelberg, Germany). Target band densities were normalized to the

Table 1 Experimental design of mouse studies 1 and 2

Group	n	Genotype
Mouse study 1		
I	8	APOE3
II	8	APOE4

Group	n	Genotype	AITC (15 mg/kg b.w.)
Mouse study 2			
I	6	APOE3	−
II	6	APOE3	+
III	6	APOE4	−
IV	6	APOE4	+

Table 2 Primer sequences of the murine (mm) and human (hs) primers for PCR analyses

Gene	Forward primer	Reverse primer
mmMT1	ACCTCCTGCAAGAAGAGCTG	GCTGGGTTGGTCCGATACTA
mmMT2	CAAACCGATCTCTCGTCGAT	AGGAGCAGCAGCTTTTCTTG
mmNrf1	GGGGACAGAATCACCATTTG	GATGCAGGCTGACATTCTGA
mmGAPDH	CCGCATCTTCTTGTGCAGT	GGCAACAATCTCCACTTTGC
mmβ-actin	GACAGGATGCAGAAGGAGATTACT	TGATCCACATCTGCTGGAAGGT
hsMT1	GCCTCTCAACTTCTTGCTTG	ACTTCTCTGATGCCCCTTTG
hsMT2	AAGAAAAGCTGCTGCTCCTG	ATCCAGGTTTGTGGAAGTCG
hsNrf1	GGGAGAATGCTGAGTTTCCA	ATGAGATCTTGCCACTGCTG
hsGAPDH	CAATGACCCCTTCATTGACC	GATCTCGCTCCTGGAAGATG
hsβ-actin	GGATGCAGAAGGAGATCACTG	CGATCCACACGGAGTACTTG

loading control TATA-binding protein (TBP) (1:500; Santa Cruz Biotechnology, Heidelberg, Germany).

^{109}Cd affinity assay

Total MT was estimated indirectly with the ^{109}Cd-haemoglobin binding assay and gamma counting as described previously (Eaton and Toal 1982).

ICP-AES

Zinc, copper and iron concentrations in liver were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, Unicam 701) as described previously (Rimbach and Pallauf 1997).

Promoter DNA methylation analysis

The presence of CpG islands within the MT1 and MT2 gene was predicted using the EMBL-EBI Open Software Suite CpGplot (http://www.ebi.ac.uk/Tools/emboss/cpgplot/). Quantitative methylation analysis of the two genes was performed with the MassARRAY® system (Sequenom, Hamburg, Germany) at Bioglobe (Hamburg, Germany) as described previously (Fischer et al. 2010). The murine H19 gene region served as control (data not shown).

Statistical analysis

The statistical analysis was performed with SPSS Version 15.0 (SPSS GmbH Software, Munich, Germany). For the comparison of two groups, tissue (Study 1) and cell culture data were tested for normal distribution (Kolmogorow-Smirnov and Shapiro-Wilk test) and analysed by Student's t-test. For the comparison of cell culture and mice data (Study 2) with more than two experimental groups, one-way ANOVA was performed following post hoc tests (Dunnett-T or Games-Howell). In the case of non-parametric data, Mann-Whitney U-test was performed. Results are expressed as means with standard errors (SEM), and significance was accepted at $p < 0.05$.

Results

In study 1, we determined the effect of the APOE genotype on tissue metallothionein, Nrf1 and Mtf1 levels as well as hepatic zinc, copper and iron concentrations in APOE3 and APOE4 targeted gene replacement mice.

Effect of APOE genotype on hepatic metallothionein

We found significantly lower mRNA steady-state levels of MT1 and MT2 in the liver of APOE4 in comparison with APOE3 mice (Fig. 1a, b). The hepatic MT protein level as measured by the ^{109}Cd affinity assay was significantly decreased in APOE4 versus APOE3 mice (Fig. 1c). Interestingly, MT1 and MT2 exhibit CpG islands in their promoter regions, suggesting putative regulatory functions through methylation in these areas. Since we found lower hepatic mRNA levels of MT1 and MT2 in APOE4 compared to APOE3 mice, we quantified the methylation status of MT1 and MT2 CpG islands in the liver of APOE4 versus APOE3 mice. The resulting epigrams revealed that all samples exhibited similar methylation patterns. We found no quantitative differences in the promoter methylation of the MT1 and MT2 gene between APOE4 and APOE3 mice (Fig. 1d).

Effect of APOE genotype on hepatic Nrf1 and Mtf1

Since gene expression of MT1 and MT2 is, at least partly, controlled by the transcription factors Nrf1 and Mtf1, we determined both mRNA and nuclear protein levels of Nrf1 and Mtf1 in the liver of both APOE genotypes. We found significantly lower Nrf1 mRNA levels (Fig. 2a) and lower nuclear Nrf1 protein levels in the liver of APOE4 versus APOE3 mice (Fig. 2b). However, no significant differences in Mtf1 mRNA and nuclear protein levels of Mtf1 were found between APOE4 and APOE3 mice (data not shown).

Hepatic zinc, copper and iron concentrations

Since transition metals, including zinc and copper, may induce metallothionein expression, we determined zinc and copper levels in the liver of our mice in response to the APOE genotype. Furthermore, zinc and copper may affect liver iron levels. However, under our study conditions, neither zinc nor copper or iron concentrations in the liver were significantly affected by the APOE genotype (Fig. 2c–e).

Effect of APOE genotype on metallothionein and Nrf1 levels in heart, kidney and lung

As differences in hepatic gene expression between APOE4 and APOE3 mice were evident, we determined MT1, MT2 and Nrf1 mRNA levels also in heart, kidney and lung in response to the APOE genotype. In contrast to APOE3 mice, MT1, MT2 and Nrf1 mRNA levels were significantly lower in heart (Fig. 3a–c), kidney (Fig. 3d–f) and lung (Fig. 3g–i) of APOE4 animals.
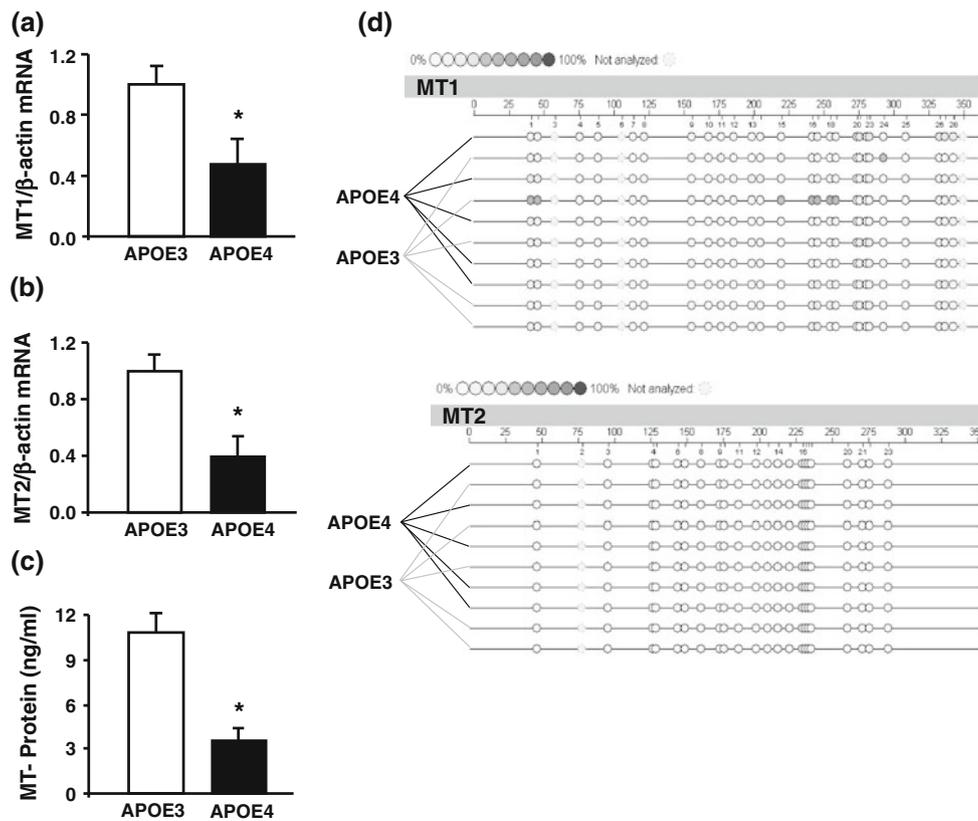
Fig. 1 Effect of APOE genotype on hepatic metallothionein 1 (MT1; **a**) and 2 (MT2; **b**) mRNA levels ($n = 8$) determined by real-time PCR as well as metallothionein protein level ($n = 8$) (**c**) as measured by ^{109}Cd affinity assay in APOE3 (*open bars*) as compared to APOE4 mice (*solid bars*). Data are means + SEM (study 1). *Asterisks* (*) indicate significant differences between APOE3 and APOE4 animals. Genomic DNA isolated from the liver of APOE3 and APOE4 mice was analysed for methylation status of cytosine-phospho-guanine (CpG) sites in the metallothionein 1 (MT1) and 2 (MT2) gene promoters (**d**). A 255-bp portion of the H19 regulatory region served as control amplicon. Exemplary data for one amplicon for each gene are shown. The *grey dots* indicate the software-determined methylation ratio at each analysed CpG unit for each sample. *Upper numbering* scheme refers to the base positions within the analysed amplicon. *Lower numbers* indicate the individual CpG sites located in the amplicon

Fig. 2 Effect of APOE genotype on hepatic Nrf1 mRNA (**a**) and nuclear protein (**b**) level in APOE3 (*open bars*) as compared to APOE4 mice (*solid bars*). Data of mRNA levels as determined by real-time PCR are means + SEM, $n = 8$ per group (study 1). *Asterisk* (*) indicates significant differences between APOE3 and APOE4 mice; effect of APOE genotype on hepatic zinc (**c**), copper (**d**) and iron (**e**) concentrations as determined by ICP-AES. Data are means + SEM, $n = 8$ per group (study 1)
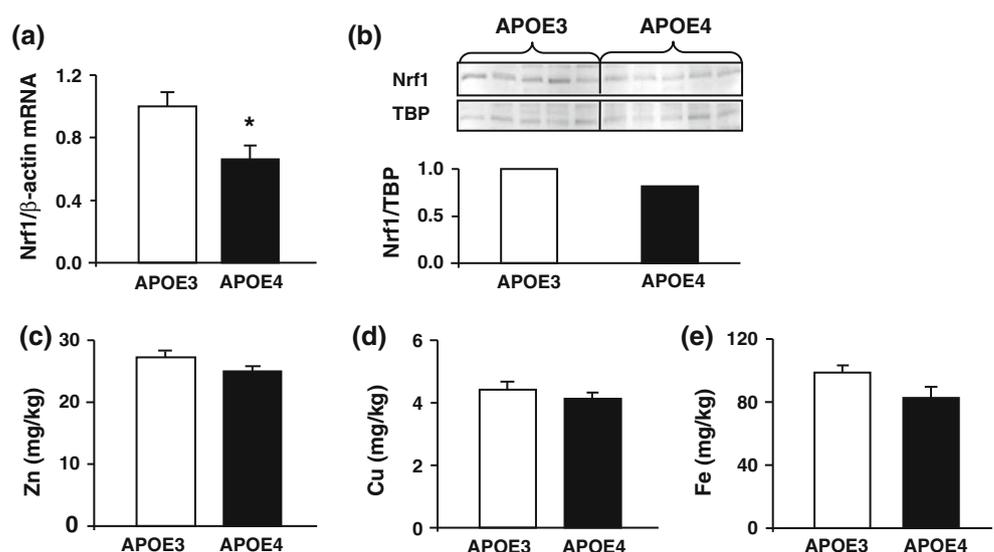
Fig. 3 Effect of APOE genotype on metallothionein 1 (MT1) and 2 (MT2) and Nrf1 mRNA levels (*n* = 8) as determined by real-time PCR in the heart (**a**–**c**), kidney (**d**–**f**) and lung (**g**–**i**) of APOE3 (*open bars*) as compared to APOE4 mice (*solid bars*). Data are means + SEM. *Asterisks* (*) indicate significant differences between APOE3 and APOE4 mice (study 1)
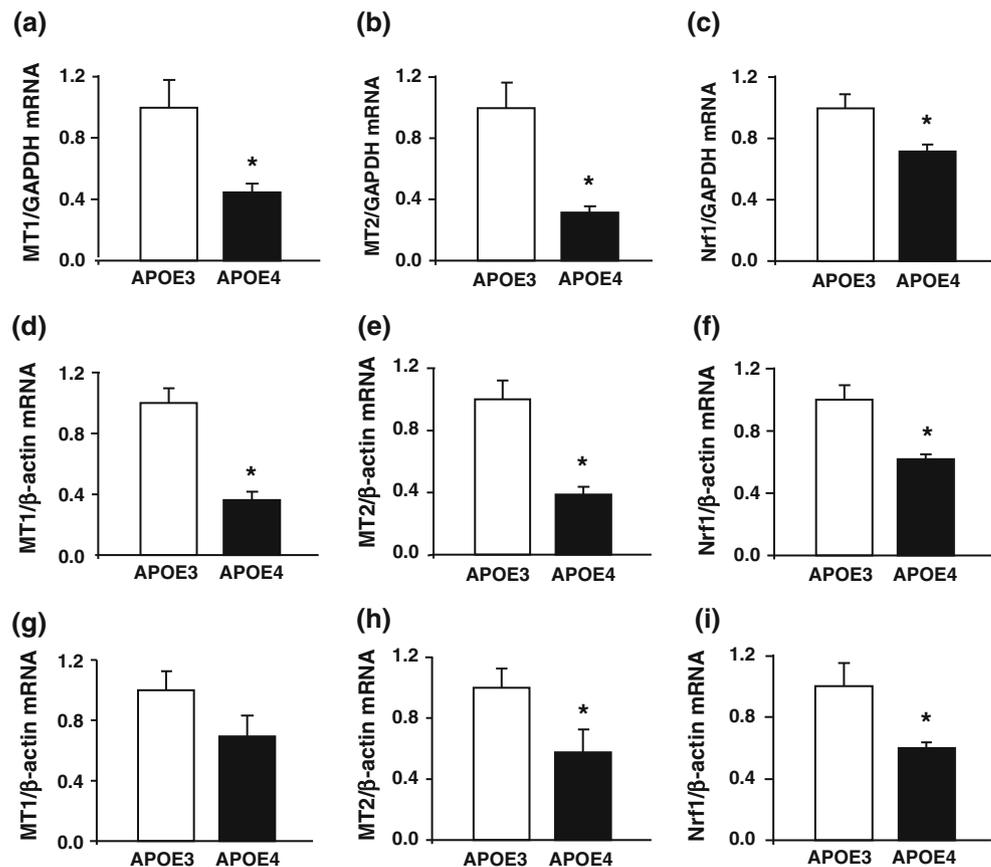
Effect of AITC on MT and Nrf1 mRNA level in Huh7 cells and liver of APOE transgenic mice

Isothiocyanates are potent inducers of metallothioneins both in cultured cells (Ernst et al. 2011) and in laboratory rodents (Hu et al. 2004). As shown in Fig. 4a, allyl-isothiocyanate (AITC) treatment of Huh7 cells at non-cytotoxic concentrations resulted in a dose-dependent increase in MT2 levels. Based on our cell culture data, we performed an additional mouse study (study 2) investigating whether dietary AITC may correct for decreased hepatic MT1, MT2 and Nrf1 mRNA levels in APOE4 mice. In agreement with mouse study 1, APOE4 mice exhibited lower hepatic MT1, MT2 and Nrf1 mRNA levels as compared to APOE3 mice in study 2 (Fig. 4b–d). Dietary AITC could only partly counteract decreased Nrf1 and MT2 but not MT1 gene expression in our APOE4 mice.

Effect of cholesterol on metallothionein and Nrf1 levels in Huh7 cells

Treatment of Huh7 cells with cholesterol (at a non-cytotoxic concentration) significantly decreased MT2 as well as Nrf1 levels in Huh7 hepatocytes as summarized in Fig. 5.

Discussion

An important finding of our study is that the APOE4 genotype, in contrast to APOE3, is associated with decreased MT levels in mice liver, heart, kidney and lung. MT exhibits antioxidant (Miles et al. 2000) and anti-inflammatory properties (Inoue et al. 2009). We have previously shown that the APOE4 genotype is associated with an altered inflammatory response (Jofre-Monseny et al. 2007) and differences in the cellular oxidant/antioxidant status (Dietrich et al. 2005; Jofre-Monseny et al. 2008a; Huebbe et al. 2007) in cultured cells, mice and humans. These differences in the inflammatory and oxidant/antioxidant status between the two genotypes may be partly related to differences in metallothionein levels.

The underlying cellular and molecular mechanisms by which APOE isoforms may affect tissue metallothionein levels are largely unknown. Our promoter DNA methylation analyses suggest that differences in MT gene expression between APOE3 and APOE4 mice are most likely not related to differences in the methylation status of both the MT1 and MT2 promoter. Furthermore, we did not observe differences in hepatic zinc and copper concentrations, both known inducers of MT, between the two genotypes. In this

Fig. 4 Effect of 10 and 25 μmol/l AITC on MT2 (**a**) mRNA level in Huh7 cells. Data are means + SEM, $n = 6$. *Asterisk* (*) indicates significant differences between untreated controls and AITC-treated cells; Effect of AITC and APOE genotype on hepatic metallothionein 1 (MT1; **b**) and metallothionein 2 (MT2; **c**) and Nrf1 (**d**) mRNA levels determined by real-time PCR in APOE3 (*open bars*) as compared to APOE4 mice (*solid bars*) fed with and without 15 mg AITC/kg body weight. Values are means + SEM, $n = 6$ (study 2). Means without a common letter significantly differ, $p < 0.05$
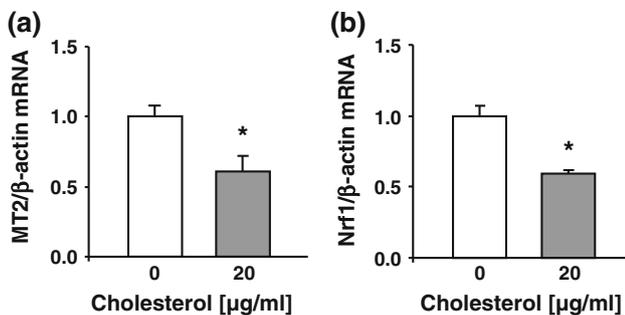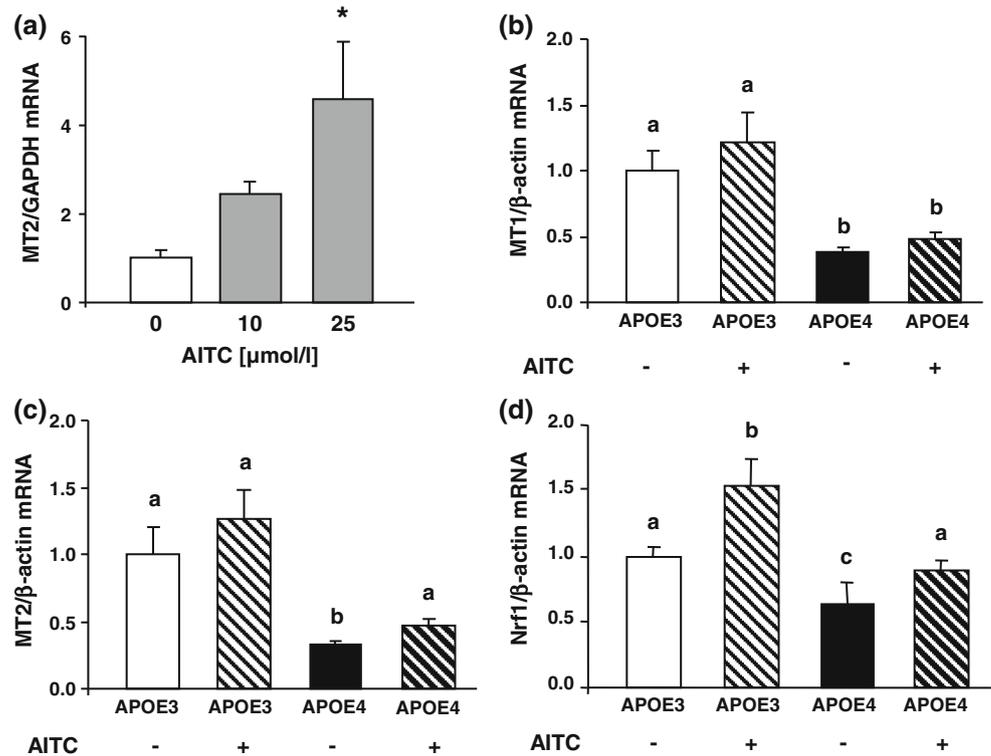
Fig. 5 Effect of cholesterol on MT2 (**a**) and Nrf1 (**b**) mRNA level in Huh7 cells. Data are means + SEM, $n = 6$. *Asterisks* (*) indicate significant differences between control and cholesterol-treated (20 μg/ml) cells

study, total liver zinc was determined by ICP-AES. However, we did not measure the free zinc pool in our mice. A recent study suggests that APOE may affect the free zinc pool since the level of histochemically reactive zinc was reduced in the brain of APOE-deficient mice compared to wild-type animals (Lee et al. 2010). Thus, future studies are warranted to test the hypothesis whether the free zinc pool may be differentially regulated in response to the APOE genotype.

We observed lower nuclear Nrf1 levels in APOE4 versus APOE3 mice. Nrf1 is an important molecular switch regulating tissue MT concentration (Ohtsuji et al. 2008). The decreased Nrf1 and MT levels in APOE4 versus

APOE3 may be due to differences in hepatic cholesterol concentrations between APOE3 and APOE4 mice. We have previously shown that APOE4 as compared to APOE3 mice exhibit elevated liver cholesterol levels (Graeser et al. 2011). Higher hepatic cholesterol levels in APOE4 mice may be due to a defective cholesterol reuptake into the hepatic tissue (Carvalho-Wells et al. 2010). Therefore, we supplemented Huh7 cells with cholesterol and determined Nrf1 and MT2 levels, which are highly expressed in this cell line. Interestingly, cholesterol significantly decreased both Nrf1 and MT2 mRNA levels (Fig. 5), indicating that differences in MT gene expression between the two genotypes could be partly related to differences in hepatic cholesterol concentrations.

Since APOE4 versus APOE3 mice exhibited lower tissue MT levels, we asked the question whether this could be corrected by dietary factors. Yeh and Yen (Yeh and Yen 2005) reported that the isothiocyanate sulforaphane (SFN) induces MT in HepG2 hepatocytes. Our recent cell culture data suggest that allyl-isothiocyanate (AITC) is less cytotoxic than SFN but almost equally potent than SFN as far its anti-inflammatory properties and the induction of stress response genes are concerned (Wagner et al. 2011). Thus, we tested AITC for its ability to induce MT2 in Huh7 hepatocytes. Similar to the effects described for SFN (Yeh and Yen 2005), we observed a dose-dependent induction of MT2 by AITC in the present cell culture study. However, in our mouse study, AITC could only partly correct for

decreased gene expression in APOE4 versus APOE3 mice. In the present study, mice were supplemented with AITC only for 1 week and only one AITC concentration was chosen. It needs to be established if long-term administration and higher doses of AITC may be more effective for inducing MT gene expression in APOE4 mice. Overall, APOE4 mice appear to be not very responsive towards Nrf1 and MT induction by AITC. This is in accordance with our previous findings in mice using flavonoids, indicating that APOE4 versus APOE3 mice exhibited lower paraoxonase-1 levels that could be better induced by dietary quercetin in APOE3 as compared to APOE4 mice (Boesch-Saadatmandi et al. 2010).

APOE4 affects both morbidity and mortality in humans (Mahley et al. 2009; Jofre-Monseny et al. 2008b). A dysregulation in MT homoeodynamics may adversely affect age-related chronic diseases (Swindell 2011). Here, we showed for the first time, a significant interaction between APOE genotype and MT levels in mice. Future studies are needed to investigate how this APOE/MT interaction affects stress response, chronic disease risk and longevity.

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