

**EFFECT OF APOLIPOPROTEIN E GENOTYPE ON NRF1 AND NRF2 DEPENDENT GENE
EXPRESSION AND TISSUE METALLOTHIONEIN LEVELS IN TARGETED GENE REPLACEMENT
MICE – ROLE OF DIETARY ALLYL-ISOTHIOCYANATE**

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The Apolipoprotein E4 genotype is an important risk factor for ageing and age-related chronic diseases, including cardiovascular and Alzheimer's disease. In contrast to the APOE3 genotype, the APOE4 genotype has been shown to be associated with increased oxidative stress and chronic inflammation. The transcription factor Nrf2 (nuclear factor erythroid-derived 2-related factor 2) regulates the expression of genes encoding for antioxidant and phase II enzymes, such as glutathione S-transferase (GST), heme oxygenase-1 (HO-1) and NAD(P)H dehydrogenase, quinone 1 (NQO1). Recent studies suggest a potential role of Nrf2 in cardiovascular disease prevention. Metallothioneins (MTs) are low molecular weight cysteine-rich proteins, which exhibit anti-oxidant and anti-inflammatory activity. It has been shown that over-expression of MTs increases life span in laboratory mice. MT gene expression is partly controlled by the transcription factor Nrf1 (nuclear factor erythroid-derived 2-related factor 1) and Nrf2. Interactions between APOE and Nrf1 as well as Nrf2 target gene expression and tissue MT levels, however, are largely unknown.

Thus this thesis aims to determine the impact of the APOE genotype on Nrf1 and Nrf2 target gene expression and tissue MT levels in mice. Present data suggest that Nrf2 dependent gene expression is affected by the APOE genotype. APOE4 compared to APOE3 mice exhibited lower hepatic protein levels of nuclear Nrf2. Furthermore mRNA and protein levels of Nrf2 target genes including GSTA2, HO-1 and NQO1 were significantly lower in APOE4 as compared to APOE3 mice. Lower hepatic mRNA levels of phase II enzymes, as observed in APOE4 vs. APOE3 mice, were accompanied by higher mRNA levels of phase I enzymes including Cyp26a1 and Cyp3a16. Furthermore miRNA-144, miRNA-125b and miRNA-29a involved in Nrf2 signalling, inflammation and regulation of phase I enzyme gene expression were affected by APOE genotype. Thus, first experimental evidence that Nrf2 is differentially regulated in response to APOE genotype is provided.

Moreover, the effect of the APOE4 vs. APOE3 genotype on MT levels in targeted gene replacement mice was determined. APOE4 mice exhibited significantly lower hepatic MT1 and MT2 mRNA as well as lower MT protein levels as compared to APOE3 mice. The decrease in hepatic MT protein levels in APOE4 as compared to APOE3 mice was accompanied by lower nuclear Nrf1 level. Cell culture experiments using Huh7 hepatocytes identified allyl-isothiocyanate (AITC) as a potent Nrf2, HO-1 and MT inducer *in vitro*. Therefore, APOE3 and APOE4 mice were supplemented with AITC. However, under the conditions investigated, AITC (15 mg/kg b.w.) could only partly counteract the decreased MT1 and MT2 gene expression in APOE4 mice *in vivo*.

Overall, present data suggest that the APOE genotype is an important determinant of Nrf1 and Nrf2 dependent gene expression and tissue MT levels in mice.