

Apolipoprotein E polymorphisms and their protective effect on Hepatitis E virus replication

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Dear Editor,

with interest, we read the report by Zhang et al. published in November 2015 entitled “Apolipoprotein E and protection against hepatitis E viral infection in American non-Hispanic blacks” (1). In a large candidate gene study of selected data from the Third National Health and Nutrition Examination Survey, 1991-1994 (NHANES III), Zhang and colleagues suggested a possible correlation between certain apolipoprotein E (ApoE) single-nucleotide polymorphisms (SNP's) and protection against hepatitis E virus (HEV) infection. Of 6272 participants from three different ethnic/racial populations in the US, they selected 497 SNP's from 190 genes potentially associated with influencing the cellular lipid metabolism and immune response upon HEV infection. In the literature three allelic isoforms of the ApoE gene are described ($\epsilon 2$, $\epsilon 3$, and $\epsilon 4$) (2). Comparing the allele frequencies of the ApoE variants and the prevalence of Anti-HEV IgG's in the cohort study, the authors provide evidence for a strong correlation of ApoE $\epsilon 3$ and ApoE $\epsilon 4$ with protection against HEV infection in non-Hispanic blacks but not in non-Hispanic whites or Mexican Americans.

To characterize the underlying mechanism of this observation, we investigated the role of ApoE SNP's on HEV replication in tissue culture. Huh-7.5 cells silenced in endogenous ApoE expression (3) were rescued to ectopically express the three ApoE allelic isoforms as determined by an ApoE-specific ELISA (Fig 1A). HEV RNA replication was analyzed by transfection of *in vitro* transcribed genotype 3 reporter replicon encoding a *Gaussia* luciferase (4). As depicted in Figure 1B, HEV replication levels were not influenced by any of the ApoE isoforms (Fig. 1B). Next, we challenged the same cell lines with genotype 3 full-length HEV RNA and determined viral replication with ribavirin as control by the detection of intracellular RNA copy numbers (Fig. 1C). Moreover, the efficiency of virus assembly and release was

determined by quantification of the accumulation of ORF2 protein in the culture fluid of transfected cells by using an ORF2-specific ELISA (5) (Fig. 1D). Importantly, HEV full-length RNA replication and virus assembly were not affected irrespective which ApoE isoform was expressed, while ribavirin treatment decreased HEV RNA replication and virus particle release (Fig. 1C and D). In summary, these results show that at least in the Huh-7.5 model, HEV RNA replication and virus production is not affected by ApoE polymorphisms, unlike the epidemiological findings reported by Zhang and colleagues.

References

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Figure legend

Figure 1: HEV replication is not influenced by expression of ApoE isoforms

Huh-7.5 3'sh ApoE KD cells were silenced of ApoE expression with a shRNA targeting the 3'-untranslated region of the human ApoE gene and rescued by ectopic expression of the three common ApoE isoforms. **A)** ApoE-specific secretion was determined by commercially available ApoE-ELISA (n = 3). **B)** Cell lines expressing different ApoE variants were transfected with HEVp6 subgenomic replicon encoding for a *Gaussia* luciferase reporter and replication was determined at 4 h, 24 h, 48 h, and 72 h post transfection by monitoring *Gaussia* activity in the supernatants. Data are depicted as relative light units (RLU) normalized to 4 h (n = 3). **C+D)** HEVp6 full length *in vitro* transcripts were transfected into cell lines expressing different ApoE variants and intracellular HEV RNA (n = 3) **(C)** as well as secreted HEV-ORF2 (n = 2) **(D)** was conducted as described and normalized to 4 h values. For each assay the median and range of the biological replicates are depicted.