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Apolipoprotein E allele frequencies in chronic and self-limited hepatitis C suggest a protective effect of *APOE4* in the course of hepatitis C virus infection

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Abstract

Background & Aims: Infectious hepatitis C virus (HCV) particles bind to host lipoproteins such as low-density lipoproteins (LDLs). Low-density lipoprotein receptors (LDLR) have been termed candidate receptors for HCV–LDL complexes. Functional host genetic single nucleotide polymorphisms (SNPs) in the apolipoprotein E (*APOE*) gene encoding apolipoprotein E (apoE) – a major structural LDL component and natural ligand of LDLR – likely influence the course of HCV infection. We investigated the prevalence of *APOE* SNPs in two large and independent cohorts of patients with chronic HCV infection compared to respective controls. *Methods:* We genotyped 996 chronically HCV-infected patients; 179 patients with spontaneous HCV clearance; 283 individuals with non-HCV-associated liver disease; and 2 234 healthy controls. *Results:* *APOE* genotype proportions in patients with persistent HCV infection significantly differed from healthy controls ($P = 0.007$) primarily because of a substantial under-representation of *APOE4* alleles in chronically HCV-infected patients (10.2%) compared to 13.0% in healthy controls ($P = 0.001$). The distribution of *APOE4* allele positive genotypes (ε2ε4, ε3ε4, ε4ε4) also significantly differed between chronically HCV-infected patients and healthy controls (1.4%, 17%, 1% vs. 2.4%, 20.5%, 1.7%; $P = 0.001$), suggesting a protective effect of the *APOE4* allele in HCV infection. This was confirmed by a significant over-representation of the *APOE4* allele in patients with spontaneous HCV clearance (17.6%; $P = 0.00008$). The *APOE4* allele distribution in patients with non-HCV-associated liver disease (14.0%) was very similar to healthy controls and also differed from chronically HCV-infected patients ($P = 0.012$), suggesting HCV specificity. *Conclusions:* Our findings suggest that the *APOE4* allele may confer a protective effect in the course of HCV infection.

Abbreviations

ALT, alanine aminotransferase; *APOE*, apolipoprotein E (genotype); ApoE, apolipoprotein E (protein); AST, aspartate aminotransferase; BMI, body mass index; GGT, γ-glutamyltransferase; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptors; SNP, single nucleotide polymorphism; VLDL, very low-density lipoproprotein.

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hepatic fibrosis – hepatic steatosis – lipoprotein – low-density lipoprotein receptor – spontaneous HCV resolution

Key points

- Infectious HCV particles bind to low-density lipoproteins (LDL). LDL receptors (LDLR) have been termed candidate receptors for HCV–LDL complexes. Functional single nucleotide polymorphisms in the apolipoprotein E *(APOE)* gene encoding apolipoprotein E (apoE) – a major structural LDL component and ligand of LDLreceptors – likely influence the course of HCV infection.
- We genotyped 996 chronically HCV-infected patients; 179 patients with spontaneous HCV clearance; and 2 234 healthy controls.
- We found a substantial under-representation of *APOE4* alleles in chronically HCV-infected patients and a significant over-representation of *APOE4* alleles in patients with spontaneous HCV clearance.
- Our findings indicate a decreased susceptibility to chronic HCV infection in *APOE4* allele carriers.

Infectious hepatitis C virus (HCV) particles bind to plasma lipoproteins such as very low-density lipoproteins (VLDLs) and low-density lipoproteins (LDLs) (1–3). Hepatic internalization of such virus–lipoprotein complexes is mediated by low-density lipoprotein receptors (LDLR) (4–6). Apolipoprotein E (protein) (ApoE), a structural component of LDLs and natural ligand of LDLR, is also integrally linked to plasma lipoprotein metabolism (7). The three major isoforms of apoE (apoE2, E3 and E4) are decoded by three alleles (*APOE* epsilon 2, 3 and 4). The most common isoform apoE3 was labelled the wild type apoE. ApoE2, E3 and E4 differ in their amino sequence at positions 112 and 158. At these different sites, apoE2 contains cysteine/cysteine, apoE3 cysteine/arginine and apoE4 arginine/arginine.

There is growing evidence that functional host-specific genetic polymorphisms within *APOE* influence on the interaction between the host and HCV, and therefore affect the natural and treatment-induced course of HCV infection (8). *APOE* single nucleotide polymorphisms have been associated with an increased likelihood of persistent HCV infection (9) and severity of consecutive histological liver damage (10). We previously associated *APOE* with treatment outcomes following standard combination therapy in chronic HCV infection (11). Other studies linked *APOE* with the risk of treatment-induced psychiatric side effects in these patients (12) or recurrent HCV infection, respectively, and liver damage following liver transplantation (13, 14). In view of these still preliminary studies being conducted on a rather small number of patients, we tried to define the role of *APOE* polymorphisms on a larger scale of patients in two independent well-characterized cohorts with chronic HCV infection, hereby correlating the different *APOE* genotypes and alleles with demographic, biochemical, molecular and available histological parameters of these patients. Patients with spontaneous HCV clearance and non-HCV-associated chronic liver disease and healthy subjects were used as respective controls.

Materials and methods

Subjects

In total, 3 692 participants gave informed consent to this study protocol, which was approved by the Ethics Committees of the Charité Berlin and the University of Leipzig; among them, two large and independent cohorts with 701 and 295 chronically HCV-infected patients; 179 patients with a history of spontaneous HCV clearance; 283 patients with non-HCV-associated chronic liver disease; and 2 234 subjects without a history of chronic liver disease. The majority of chronically HCV-infected patients had been enrolled in prospective treatment studies at three different liver centres, comprising the Universities of Berlin, Frankfurt/Main and Homburg in Germany. The diagnosis of chronic hepatitis C was based on the elevated serum transaminase levels for at least 6 months and consistently detectable serum HCV–RNA. All patients were anti-HCV positive and negative for hepatitis B surface antigen and antibodies to human immunodeficiency virus 1 and 2. Quantitative assessment of serum RNA was performed either by a standardized quantitative reverse transcription-polymerase chain reaction assay (Amplicor Monitor HCV™ version 2.0; Roche Diagnostic system, Roche Diagnostics, Penzberg, Germany) or by the third-generation branched DNA assay (Versant Quantitative HCV RNA 3.0 Assay; Bayer Diagnostics, Emeryville, CA, USA). HCV–RNA levels were standardized to International Units per millilitre (IU/ml) according to the formula described by Pawlotski *et al.* (15). Genotyping of HCV was performed by reverse hybridisation assay (Inno LiPA HCV II, Innogenetics, Gent, Belgium). Table 1 summarizes all relevant demographic and biochemical data of the initial study cohort with 701 chronically HCV-infected patients which were assessed prior to therapy. Table 1 also depicts the liver histology from 555 liver biopsies (79%) which were available from this cohort. Hepatic inflammation (grade) and fibrosis (stage) were classified according to the semiquantitative histological score described by Scheuer *et al.* (16). Exact percentage of fatty degener-

Table 1. Relevant demographic, biochemical, molecular and histological data of 701 study participants with chronic hepatitis C infection

	Patients with chronic hepatitis C	
Demography		
Male/female (% male)	391/310	(56%)
Age (years)*	48.7 ± 0.31	(19–82)
Risk factors for hepatitis C infection (%)		
Intravenous drug abuse	145	(20.7%)
Recipient of blood products	165	(23.5%)
Others†	30	(4.3%)
Sporadic/unknown	361	(51.5%)
Biochemistry‡		
ALT	62.4 ± 2.0	(6–422)
AST	36.5 ± 0.9	(8–329)
AP	115.7 ± 1.9	(12–853)
GGT	43.5 ± 2.3	(4–341)
Ferritin	221.7 ± 11.1	(6–2 230)
Plasma cholesterol	178.7 ± 2.3	(79–355)
HCV genotype (1; 2; 3; 4; 6; not determined)	434; 52; 154; 16; 2; 43	
Viremia ($\times 10^6$ IU/ml)*	2.9 ± 0.3 (0.008–98)	
Liver histology§		
Inflammation (grade; n = 555)		
Absent	2	(0.4%)
Minimal	14	(2.5%)
Mild	187	(33.7%)
Moderate	277	(49.9%)
Severe	75	(13.5%)
Fibrosis (stage; n = 555)		
Absent	22	(4.0%)
Mild without septa	176	(31.7%)
Moderate with few septa	192	(34.6%)
Numerous septa, no cirrhosis	121	(21.8%)
Cirrhosis	44	(7.9%)
Steatosis (%; n = 215)¶		
None	39	(18.1%)
<25	78	(36.3%)
25–50	65	(30.2%)
>50	33	(15.3%)

*Mean ± SEM (range).

†Including occupational exposure, sexual transmission, skin tattoos, treatment for bilharziosis.

‡Upper normal limit of laboratory data (ULN, measured at 25°C): ALT, 19 U/L (female), 23 U/L (male); AST, 18 U/L (female), 21 U/L (male); AP, 60–180 U/L (female/male), GGT, 18 U/L (female); 28 U/L (male); Ferritin, 10–200 μg/L (female), 30–300 μg/L (male); cholesterol, 200 mg/L (female/male)

§Histological classification according to the Scheuer score (16).

¶Only histological reports with notification of the exact percentage of fatty degenerated hepatocytes were considered.

ated hepatocytes obtained from 430 histological reports (31%) was classified according to the following scheme: 0% (none), <25%, 25–50% and >50% fatty degenerated hepatocytes.

The validation cohort with 295 chronically HCV-infected comprised 161 males (54.6%) and 134 women (45.4%). Spontaneous HCV clearance was assumed by the presence of positive HCV antibody titres but persistent negative HCV–RNA (PCR) and normal transaminase levels during the preceding 6 months. Of the group of 179 individuals with self-limited HCV infection, 75 (41.9%) were male and 94 (52.5%) were female. Of the group of 283 patients with non-HCV-associated chronic liver disease, 129 (45.6%) were male and 154 (54.4%) were female; among them, patients with chronic hepatitis B infection, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis and alcoholic liver disease. The controls investigated were blood donors from South-West and East Germany. In total, we screened 2 234 controls (932 male, age range 18–81 years, median 50 years).

Apolipoprotein genotyping and statistical analysis

Commercially available kits (Qiagen, Hilden, Germany) were used to extract the DNA from EDTA blood samples. *APOE* genotyping was performed by restriction fragment length polymorphism analysis as described (17). For analysis of the control subjects, we performed melting curve analysis in a LightCycler 480 instrument (Roche Diagnostics). PCR was performed using 0.75 U AmpliTaq Gold polymerase (Applied Biosystems, Inc. Foster City, CA, USA), 400 μM dNTPs, 1.5 mM $MgCl_2$ and 0.4 μM F-Primer as well as 0.1 μM R-primer in a total volume of 25 μl. Cycle conditions were an initial denaturation for 12 min at 95°C followed by 47 cycles of 20 s denaturation at 95°C, 40 s annealing at 64°C, 90 s primer extension at 72°C and a final extension for 2 min at 72°C in an automated thermal cycler (Applied Biosystems). Primers were synthesized according to the published nucleotide sequences (GenBank: NC_000 019.9): F-Primer 5′-TTGAAGGCCTACAAATCGGAAC TG-3′; R-Primer 5′-GCCCCGGCCTGGTACAC-3′.

We performed melting curve analysis using two pairs of fluorescent resonance energy transfer (FRET) probes. FRET probes were designed and synthesized by TIB Molbiol (Berlin, Germany): Codon 112: Sensor probe 5′-LC640-ACATGGAGGACGTGCGCGG-3′, Anchor probe 5′-CTGCAGGCGGCGCAGGCCCGGCTGGGCG C-FL-3′; Codon 158: Sensor probe 5′-LC610-GACCTG CAGAAGCGCCTGGC-3′, Anchor probe 5′-GCTGCGT AAGCGGCTCCTCCGCGATGCCG-FL-3′. Analytical melting included the following steps: 97°C for 60 s, 45°C for 60 s and an increase to 80°C at a 0.19°C/s ramp rate.

Corresponding *APOE* allele frequencies are displayed as their group percentages. Possible implications of *APOE* in the course of the HCV infection are expressed by odds ratios (OR) with a 95% confidence limit (CI). Nonparametric data are expressed as median (range). Statistical analysis was performed by contingency tables using ^{2}א statistics and Mann–Whitney tests. All statistical calculations were performed with SPSS 11.0 software for Windows (SPSS, Inc, Chicago,

IL, USA). If not stated otherwise, all tests were two-sided and *P*-values lower than 0.05 were considered significant.

Results

The distribution of *APOE* genotypes and allele frequencies differs in patients with chronic hepatitis C and control subjects without chronic liver disease

In total, 701 patients with chronic HCV infection and an independent validation cohort comprising 295 additional patients with chronic HCV infection and 2 234 control subjects without chronic liver disease were included in this study. The overall distribution of *APOE* genotypes and corresponding allele frequencies in the two HCV cohorts and the control subjects are depicted in Table 2. There was no difference in the *APOE* genotype distribution between the initial HCV cohort and the validation cohort (Table 2), which were therefore combined for the subsequent statistical analysis. *APOE* genotypes in HCV patients and controls were in Hardy–Weinberg equilibrium, but the overall distribution of *APOE* genotypes and *APOE* alleles differed significantly between the two groups ($P < 0.007$; data not shown). This was because of a marked over-representation of the *APOE3* allele in patients with chronic hepatitis C (82.7% *APOE3*) compared to 78.6% *APOE3* in control subjects (OR 1.052; 95% CI 1.026–1.079; $P < 0.0002$, Table 2), confirming an earlier study which already associated the *APOE3* allele with an increased risk for persistent HCV infection (9). However, our data also revealed a substantial under-representation of the *APOE4* allele in patients with chronic hepatitis C (10.5% *APOE4*) compared to 13.0% *APOE4* in control subjects (OR 0.780; 95% CI 0.671–0.906; $P = 0.001$, Table 2), suggesting a protective effect of *APOE4* in the course of HCV infection. Consistent with this observation, subjects carrying at least one *APOE4* allele (*APOE4*+ genotypes: ε2 ε4, ε3 ε4, ε4 ε4) were significantly under-represented in chronically HCV-infected patients (19.4% *APOE4*+) compared to 24.5% *APOE4*+ in control subjects (OR 0.791; 95% CI 0.684–0.916; $P = 0.001$, Table 2).

Association of *APOE4* with self-limited HCV infection

Genotyping of 179 patients with self-limited HCV infection revealed a significant over-representation of the *APOE4* allele (17.6% *APOE4*) compared to chronically HCV-infected patients (OR 1.727; 95% CI 1.332–2.238; $P < 0.00009$, Table 3) and control subjects (OR 1.346; 95% CI 1.063–1.706; $P = 0.016$, data not shown). *APOE4*+ genotypes were also significantly over-represented in the group of patients with spontaneous HCV resolution (34.1% *APOE4*+) compared to chronically HCV-infected patients (OR 1.759; 95% CI 1.384–2.235, $P < 0.00002$, Table 3) and control subjects (OR 1.392; 95% CI 1.121–1.728, $P = 0.005$, data not shown), providing further evidence of an increased likelihood of a self-limited HCV infection. To exclude any sampling effects because of the independence of the HCV patients and the control subjects, and to confirm the HCV specificity of our present observation, we also genotyped 283 patients at comparable risk for HCV exposure but with non-HCV-associated chronic liver disease. These patients were presented with an *APOE* genotype and allele distribution (26.9% *APOE4*+ respectively 14% *APOE4* allele, Table 3) which was very similar to the control group ($P = 0.421$ for *APOE4*+ respectively $P = 0.598$ for *APOE4*, data not shown). However, the *APOE4*+ genotype and the *APOE4* allele distribution in 283 patients with non-HCV-associated chronic liver disease also significantly differed from chronically HCV-

Table 2. *APOE* genotype proportions in two independent cohorts of patients with chronic hepatitis C and healthy controls

APOE	Chronic hepatitis C cohort-1 (n = 701)		Chronic hepatitis C cohort-2 (n = 295)		*P*	Healthy controls (n = 2 234)		OR	95% CI	*P*
Genotype										
ε2 ε2	1	(0.1%)	1	(0.3%)	n.s.	11	(0.5%)			n.s.
ε2 ε3	80	(11.4%)	44	(14.9%)	n.s.	299	(13.4%)			n.s.
ε2 ε4	7	(1.0%)	7	(2.4%)	n.s.	53	(2.4%)			n.s.
ε3 ε3	480	(68.5%)	197	(66.8%)	n.s.	1 377	(61.6%)	1.103	1.045–1.164	0.001
ε3 ε4	126	(18.0%)	43	(14.6%)	n.s.	457	(20.5%)	0.829	0.707–0.973	0.021
ε4 ε4	7	(1.0%)	3	(1.0%)	n.s.	37	(1.7%)			n.s.
APOE4+*	140	(20.0%)	53	(18.0%)	n.s.	547	(24.5%)	0.791	0.684–0.916	0.001
APOE4−†	561	(80.0%)	242	(82.0%)		1 687	(75.5%)			
Allele										
ε2	89	(6.3%)	53	(9.0%)	0.045	374	(8.4%)			n.s.
ε3	1 166	(83.2%)	481	(81.5%)	n.s.	3 510	(78.6%)	1.052	1.026–1.079	0.0001472
ε4	147	(10.5%)	56	(9.5%)	n.s.	584	(13.0%)	0.780	0.671–0.906	0.001

*Study participants carrying the apolipoprotein E4 allele: *APOE4*+: ε2 ε4, ε3 ε4, ε4 ε4.
†Study participants without the apolipoprotein E4 allele: *APOE4*−: ε2 ε2, ε2 ε3, ε3 ε3.
APOE, apolipoprotein E (genotype), CI, confidence limit; OR, odds ratios.

Table 3. Comparison of *APOE* proportions in patients with chronic hepatitis C, spontaneous HCV clearance and non-HCV-associated chronic liver disease

APOE	Chronic hepatitis C cohort-1 + 2 (*n* = 996)		Spontaneous HCV clearance (*n* = 179)		OR	95% CI	*P*	Non-HCV liver disease (n=283)		OR	95% CI	*P*
Genotype												
ε2ε 2	2	(0.2%)	0	(0.0%)			n.s.	0	(0.0%)			n.s.
ε2 ε3	124	(12.5%)	25	(14.0%)			n.s.	29	(10.2%)			n.s.
ε2 ε4	14	(1.4%)	8	(4.5%)	3.180	1.354–7.469	0.012	6	(2.1%)			n.s.
ε3 ε3	677	(67.9%)	93	(52.0%)	0.764	0.660–0.886	0.00004199	178	(62.9%)			n.s.
ε3 ε4	169	(17.0%)	51	(28.5%)	1.679	1.282–2.199	0.0004189	67	(23.7%)	1.395	1.086–1.792	0.012
ε4 ε4	10	(1.0%)	2	(1.1%)			n.s.	3	(1.1%)			n.s.
*APOE4+**	193	(19.4%)	61	(34.1%)	1.759	1.384–2.235	0.00001959	76	(26.9%)	1.386	1.101–1.745	0.008
APOE4−†	803	(80.6%)	118	(65.9%)				207	(73.1%)			
Allele												
ε2	142	(7.1%)	33	(9.2%)			n.s.	35	(6.2%)			n.s.
ε3	1 647	(82.7%)	262	(73.2%)	0.885	0.829–0.945	0.0000334	452	(79.9%)			n.s.
ε4	203	(10.2%)	63	(17.6%)	1.727	1.332–2.238	0.00008304	79	(14.0%)	1.37	1.075–1.746	0.012

*Study participants carrying the apolipoprotein E4 allele: *APOE4+*: ε2 ε4, ε3 ε4, ε4 ε4.
†Study participants without the apolipoprotein E4 allele: *APOE4−*: ε2 ε2, ε2 ε3, ε3 ε3.
APOE, apolipoprotein E (genotype); CI, confidence limit; OR, odds ratios.

infected patients (OR 1.386; 95% CI 1.101–1.745; $P = 0.008$ for *APOE4+* genotype; respectively OR 1.370; 95% CI 1.075–1.746; $P = 0.012$ for the *APOE4* allele, Table 3).

Comparison of relevant clinical and histological parameters in chronically HCV-infected *APOE4+* and *APOE4−* patients

The comparison of relevant clinical and histological data in *APOE4+* and *APOE4−* patients with chronic HCV infection revealed that only total plasma cholesterol levels differed significantly between these two groups, showing higher mean cholesterol levels in *APOE4+* allele carriers (Table 4 and Fig. S1). Table 5 compares characteristic histological findings in chronically HCV-infected patients with respect to their *APOE4* allele carrier status. In patients with mild liver disease (i.e. inflammation grade ≤1 or fibrosis stage ≤1), a significant higher *APOE4* allele proportion was found. This association was even more pronounced when analysis was restricted to HCV type 1-infected patients (14% *APOE4* allele frequency in inflammation grade ≤1 vs. 9.1% in grade >1; OR 0.57, 0.36 –0.9, $P = 0.016$; data not shown). However, when we compared the *APOE4* allele status of all patients with more advanced liver disease (i.e. inflammation grade >2 or fibrosis stage >2), no significant differences in the *APOE4* allele frequency could be observed. The degree of liver steatosis in *APOE4* allele carriers was significantly reduced compared to patients without *APOE4* allele.

Table 4. Relationship between *APOE4* allele carrier status and demographic and biochemical characteristics in 701 patients with chronic hepatitis C

Parameters*†	*APOE4(−)*	*APOE4(+)*	*P*
Age	48.6 ± 0.3	49.2 ± 0.9	0.461
BMI	24.9 ± 0.2	24.5 ± 0.4	0.816
ALT	63.3 ± 1.5	54.6 ± 3.3	0.129
AST	36.8 ± 0.9	34.2 ± 3.2	0.112
GGT	44.0 ± 1.7	38.6 ± 4.0	0.556
Ferritin	226.3 ± 12.2	186.8 ± 22.2	0.409
Total cholesterol	177.5 ± 1.7	189.1 ± 4.4	0.009
Viremia ($\times 10^6$ IU/ml)	2.9 ± 0.2	3.0 ± 0.4	0.232

*Mean ± SEM (range).
†Upper normal limit of laboratory data (ULN, measured at 25°C): ALT, 19 U/L (female), 23 U/L (male); AST, 18 U/L (female), 21 U/L (male); AP, 60–180 U/L (female/male), GGT, 18 U/L (female); 28 U/L (male); Ferritin, 10–200 μg/L (female), 30–300 μg/L (male); total cholesterol, 200 mg/L (female/male).
ALT, alanine aminotransferase; *APOE*, apolipoprotein E (genotype); AST, aspartate aminotransferase; BMI, body mass index; GGT, γ-glutamyltransferase.

Discussion

This study investigated the role of *APOE* polymorphism in two large and independent cohorts of 701 and 295 chronically HCV-infected patients. The under-representation of *APOE4* allele carriers in patients with chronic hepatitis C compared to the over-representation of *APOE4* allele carriers in patients who spontaneously cleared HCV infection suggests a protective role of *APOE4* in the course of HCV infection. This interpretation is further supported by the fact that the observed frequency of *APOE4* allele-carrying patients ($n = 140$) differed significantly from predictions by the Hardy–Weinberg equation ($n = 158$ expected) whereas the *APOE* genotype distribution in our control group was comparable to data published for the general population

Table 5. Relationship between *APOE4* allele carrier status and histological damage in 555 patients with chronic hepatitis C

	APOE4 allele	OR	95% CI	*P*
Inflammation (grade)*				
<1	13.5			
>1	9.2	0.43	0.44–0.95	0.027
<2	11.1			
3	8.7	0.76	0.41–1.38	0.365
Fibrosis (stage)*				
<1	13.4			
>1	9.4	0.67	0.46–0.98	0.041
≤2	11.4			
>2	9.4	0.81	0.52–1.24	0.323
Steatosis (%)†				
<25	14.4			
>25	8.2	0.53	0.29–1.0	0.050
None	17.9			
Steatosis	9.3	0.47	0.24–0.91	0.025

*Histological classification according to the Scheuer score (16).

†Only histological reports with notification of the exact percentage of fatty degenerated hepatocytes were considered.

APOE, apolipoprotein E (genotype); CI, confidence limit; OR, odds ratios.

(18–20). When we compared our findings with epidemiological data obtained from two other German cities [1000 subjects in Münster (21) and 1031 subjects in Marburg (22)], the clear under-representation of patients with at least one *APOE4* allele (E4+) in our group of chronically HCV-infected patients was almost very similar or even more pronounced (140 E4+ patients with chronic hepatitis C vs. 255 E4+ subjects in the Marburg group, OR 0.73, 95% CI 0.58–0.92, $P < 0.008$ vs. 280 E4+ subjects in the Münster group, OR 0.67, 95% CI 0.53–0.84, $P < 0.0006$, data not shown). It may be of interest to add that *APOE4* analysis in 283 non-HCV-infected patients with chronic liver disease (Table 3) revealed no significant differences compared to the control group (data not shown).

To understand the suggested protective function of the *APOE4* allele, insight into the complex relationship between HCV infection and the lipid metabolism within the human host is crucial (23). Thus, infectious HCV particles can bind to plasma lipoproteins such as LDL and VLDL which enables them to interact with the ApoB/E-LDL receptor on hepatocytes. It is believed that this mechanism promotes HCV cell entry (3–6). Moreover, the association of HCV E2 with LDL has been reported to enhance hepatocellular binding of LDL (24) and therefore might contribute to the epidemiological observation of decreased serum cholesterol levels in chronically HCV-infected patients. The cellular ApoB/E receptor expression itself is regulated by the human serum lipoprotein concentration. Thus, on the one hand, *APOE* polymorphism could exert an indirect effect on LDL receptor expression as increased binding and internalization of lipoproteins, which was demonstrated for *APOE4* allele carriers (25, 26), is thought to cause downregulation of cellular LDL receptors with subsequent hyperbetalipoproteinemia and hypercholesterolaemia (27–30). This association of increased cholesterol levels in *APOE4* carriers was also demonstrated in our *APOE4*-carrying population with chronic hepatitis C. On the other hand, the *APOE4*-induced hyperbetalipoproteinemia could directly interfere with the LDL receptor-mediated cellular virus uptake due to enforced competition between free betalipoproteins and virus–lipoprotein particles for free LDL receptor sites (5). Thus, these functional properties in lipid metabolism related to *APOE* polymorphism provide a reasonable explanation that *APOE4*-related downregulation of LDL receptors can exert a protective effect on HCV infection. Moreover, ApoE protein levels, which have been shown to mediate evasion from hepatitis C-neutralizing antibodies in a recent study (31), appear to be the lowest in *APOE4* allele carriers (19, 32). Therefore, HCV infection might be less likely to evade neutralizing antibodies in these patients.

Several limitations of the present candidate gene study must be acknowledged. First, previous genome-wide association studies could not confirm a major role of *APOE* in the course of HCV infection. Second, chronic HCV infection was also found in homozygous *APOE4* allele carriers in the present study. Taken together, the protective effect of *APOE4* might be rather limited as compared to the well-established effect of IL28B polymorphisms. IL28B genotyping was available in 751 patients with chronic HCV infection and *APOE* was not in linkage disequilibrium with the IL28B rs12979860 polymorphism. However, it was interesting to note that we detected the lowest proportion of *APOE4* allele carriers in patients with a history of intravenous drug abuse (7.2% vs. 11.3%, $P = 0.045$; data not shown), suggesting that *APOE4* might be relevant in the case of low viral contamination, i.e. in HCV-contaminated needle sharing.

Another interesting observation made by other groups (18, 19, 33, 34) may deserve further attention in the future. These authors pointed out that there exists a clear decreasing north–south gradient of the *APOE4* allele frequency through European countries which is inversely correlated with the prevalence of HCV infection (35). The *APOE4* allele was also identified as a strong genetic risk factor for the development of Alzheimer's disease in patients suffering from HSV infection (36). Chronic liver disorders of different aetiologies have been also studied in this respect. Corpechot *et al.* (37) discussed that patients with primary biliary cirrhosis may have a more severe course of the disease and are also less sensitive to therapeutic interventions when carrying the *APOE4* allele. In contrast to the observation by Wozniak *et al.* (10), *APOE4* allele frequencies in our chronic hepatitis C study population did not differ significantly with respect to the severe fibrosis stage (≤2 vs.

>2) or inflammation grade (≤2 vs. 3). One reason for these discrepant findings may be that different scores for histological classification were used in these studies (Knodell score vs. Scheuer score). One should also point out that numbers of patients varied greatly in these two studies. However, in our analysis comparing *APOE4* vs. non-E4 allele carriers we could indeed observe a higher proportion of *APOE4* allele carriers in patients with documented mild fibrosis or inflammation (stage and grade ≤1).

In conclusion, the under-representation of *APOE4* allele carriers in patients with chronic hepatitis C compared to the over-representation of *APOE4* allele carriers in patients who spontaneously cleared HCV infection suggests a protective role of the *APOE4* allele in the course of HCV infection. These *APOE4* allele-related data give, for the first time, a convincing hint that this allele is not only associated with negative clinical aspects as for instance increased risk for atherosclerosis and Alzheimer's disease but may also convey protective genetic functions.

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