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Apolipoprotein-E and hepatitis C lipoviral particles in genotype 1 infection: Evidence for an association with interferon sensitivity

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See Editorial, pages 3–5

Background & Aims: Hepatitis C virus (HCV) interacts with apolipoproteins B (apoB) and E (apoE) to form infectious lipoviral particles (LVP). Response to peginterferon is influenced by interferon-stimulated genes (ISGs) and *IL28B* genotype. LDL cholesterol (LDL-C) also predicts interferon response, therefore we hypothesised that LVP may also be associated with interferon sensitivity.

Methods: LVP (HCV RNA density $\leqslant$1.07 g/ml) and 'non-LVP' (d >1.07 g/ml) were measured in 72 fasted HCV-G1 patients by iodixanol density gradient ultracentrifugation and the LVP ratio (LVP/LVP + non-LVP) was calculated. Fasting lipid profiles and apolipoproteins B and E were measured. Interferon-gamma-inducible protein 10 kDa (IP10), a marker of ISGs, was measured by ELISA.

Results: Complete early virological response (EVR) was associated with lower apoE (23.9 ± 7.7 *vs.* 36.1 ± 15.3 mg/L, $p = 0.013$), higher LDL-C ($p = 0.039$) and lower LVP ratios ($p = 0.022$) compared to null responders. In multivariate linear regression analysis, apoE was independently associated with LVP (R^2 19.5%, $p = 0.003$) and LVP ratio ($p = 0.042$), and negatively with LDL-C ($p < 0.001$). IP10 was significantly associated with ApoB ($p = 0.001$) and liver stiffness ($p = 0.032$). *IL28B* rs12979860 CC was associated with complete EVR ($p = 0.044$), low apoE (CC 28 ± 11 *vs.* CT/TT 35 ± 13 mg/L, $p = 0.048$) and higher non-LVP ($p = 0.008$). Logistic regression analysis indicated that patients with high LVP ratios were less likely to have EVR (odds ratio 0.01, $p = 0.018$).

Conclusions: In HCV-G1, interferon sensitivity is characterised by low LVP ratios and low apoE levels in addition to higher LDL-C and *IL28B* rs12979860 CC. Null-response is associated with increased LVP ratio. The association of apoE and LVP with peginterferon treatment response suggests that lipid modulation is a potential target to modify interferon sensitivity.

Introduction

Hepatitis C virus (HCV) interacts with lipids at multiple stages in its lifecycle, including replication on lipid droplets [1], assembly [2], release and viral entry [3,4]. A variable fraction of HCV in serum is associated with triglyceride-rich lipoproteins (TRLs), such as very-low-density-lipoprotein (VLDL), that contain apolipoprotein B (apoB) and apolipoprotein E (apoE). These particles have been termed lipoviral particles (LVP) [5] and exhibit increased infectivity *in vitro* [1] and in animal models [6]. We have developed a novel assay to measure LVP in patients using iodixanol density gradient ultracentrifugation and demonstrated an association of LVP (density $\leqslant$1.07 g/ml) in the fasting state with insulin resistance, triglycerides and lack of early virological response in HCV genotype-1 (HCV-G1) infection [7]. Furthermore, after a high fat meal, the quantity of HCV RNA detected in a very-low-density fraction (d <1.025 g/ml) increases on average 26-fold. These very low density HCV particles are derived from both *de novo* production and intravascular transfer, and are then rapidly cleared [8,9].

Clearance of TRLs (e.g. VLDL and chylomicrons) from the plasma is facilitated by apoE [10]. ApoE exists in three common isoforms (E2, E3, and E4) that affect LDL receptor binding and modulate innate and adaptive immune responses to lipid antigens [11]. ApoE is now recognised as a central component of

Keywords: Cholesterol; Apolipoprotein B; CXCL10; Interferon stimulated genes; *IL28B* genotype.

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Abbreviations: apoE, apolipoprotein E; HCV, hepatitis C virus; LVP, lipoviral particles; apoB, apolipoprotein B; EVR, early virological response; VLDL, very-low-density-lipoprotein; TRL, triglyceride-rich lipoprotein; LDL, low-density-lipoprotein; IP10, interferon gamma-inducible protein-10 kDa; ISG, interferon stimulated genes.

the HCV–host lipid interaction through involvement at several stages in the viral lifecycle, including intracellular viral assembly [2,12–14] and as a surface constituent of secreted LVP *in vivo* and *in vitro* [15,16]. Electron microscopy indicates that infectious LVP are enriched in apoE on the surface [17], implicating apoE in mediating HCV infectivity via lipoprotein receptors [18,19].

The discovery that interleukin 28B (IL28B) single nucleotide polymorphisms (SNPs) are the most important genetic determinants of chronicity [20] and response to anti-viral therapy in HCV-G1 infection [21–25] suggests that previous associations of apoE genotype and clinical outcomes in HCV-G1 need to be re-evaluated [19–21]. The innate immune response is a critical determinant of clinical outcomes that is mediated through expression of interferon stimulated genes (ISGs). It is a paradox that pegylated interferon-α (PegIFNα) is the mainstay of treatment but activation of ISGs and high pre-treatment endogenous interferons are associated with non-response to anti-viral therapy [26–28]. Restoration of interferon sensitivity remains a target to minimise resistance to direct acting anti-viral (DAA) drugs in combination therapy [29]. The *IL28B* non-response alleles have been associated with increased ISG expression [28,30,31]. Interferon-γ-inducible-protein-10 kDa (IP10/CXCL10) is a circulating ISG product and a quantifiable serum marker for overall ISG activation. IP10 levels are associated with treatment outcome [30] and may improve the predictive value of *IL28B* genotype for individualised treatment [31]. We and others have previously reported that low apoB-associated cholesterol (i.e. LDL/non-HDL cholesterol) is an independent determinant of sustained virological response (SVR) in HCV-G1 infection [32–34], and recent data indicates that this remains important in treatment experienced patients treated with DAAs [35], suggesting that lipid pathways may also be affected by ISGs [36].

We hypothesised that interferon sensitivity was also associated with LVP. We therefore evaluated LVP in HCV-G1 patients with evidence of interferon sensitivity who achieved early virological response (EVR) compared to null-responders and those with the favourable *IL28B* rs12979860 CC genotype compared to the unfavourable CT/TT genotypes. ApoE was measured because of its central role in the HCV–host interaction, and IP10 was measured because of its association with interferon sensitivity and a marker of endogenous ISGs.

Materials and methods

Patients

Patients aged >18 years with HCV-G1 infection, including both treatment naïve and previous non-responders, attended for a morning fasting blood test. Individuals that were alcohol dependent, on concurrent lipid lowering or anti-viral therapy or co-infected with hepatitis B or human-immunodeficiency virus, were excluded. Seventy-two patients provided written informed consent to participate. Patients (27/72) returned for a second fasting blood test within 8 weeks, with no direct intervention between visits. This group was used to independently confirm any associations identified from the primary analysis. Patients were recruited jointly from two centres (Newcastle upon Tyne Hospitals NHS Foundation Trust and Imperial College London Healthcare Trust). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by both institutions ethics committees (REC 07/H0902/45; REC 07/S0501/21).

Clinical and laboratory assessment

Total cholesterol, triglyceride, and HDL-cholesterol (HDL-C) were measured by standard automated enzymatic methods using an Olympus AU640 analyser (Olympus, Watford, UK). LDL cholesterol (LDL-C) was calculated using the Friedewald equation [37]. Apolipoprotein A–I, apoB, and apoE were measured on each sample by automated rate nephelometric methods (BNII, Dade Behring Marburg GmbH, Marburg, Germany). Patients had assessment of liver stiffness using transient elastography (FibroScan®) within 8 weeks from the time of the fasting blood sample.

Measurement of HCV–LVP

Fasting plasma was fractionated by density using 12.5% self-forming iodixanol density gradient ultracentrifugation as described previously [7]. The LVP fraction was defined as HCV RNA detected in the top 3.5 ml at density $\leqslant$1.07 g/ml. The non-LVP fraction was defined as HCV RNA detected at density >1.07 g/ml in the bottom 6.5-ml fraction. The LVP ratio was calculated as: LVP/(LVP + non-LVP).

Measurement of IP10

Serum IP10 levels were measured using an ELISA kit (R&D Systems, Abingdon, Oxon, UK) according to the manufacturer's instructions. Samples were run in duplicate and absorbencies were read at OD_{450nm} with wavelength correction at OD_{570nm}. Serum IP10 concentration was calculated against a standard curve using the regression equation. A coefficient of variation (CV) value was also calculated for each duplicate set of readings from the mean/standard deviation.

ApoE and IL28B genotyping

ApoE genotype was determined using an automated dual fluorescent melting curve technique on genomic DNA isolated from whole blood using a commercial kit (Nucleon DNA extraction kit, Amersham Life Sciences, UK) in 71 patients with 1 genotyping failure in the remaining patient.

Patients (55/72) provided consent for stored DNA to be used for *IL28B* genotyping. Genomic DNA was isolated from 2 ml of whole blood using QIAamp midi kits (QIAGEN, Crawley, UK). The SNP rs12979860 in the *IL28B* gene was genotyped independently by KBiosciences (Hoddesdon, Hertfordshire, UK) using the KASPar chemistry SNP genotyping system using FRET quencher cassette oligonucleotides (http://www.kbioscience.co.uk). Genotyping for rs12979860 failed in four samples.

Statistical analysis

Continuous data was assessed by normality tests and summarised by the mean ± standard deviation if normally distributed. Two sample t-tests were used for comparison of normal variables between *IL28B* genotypes, and one-way ANOVA for comparison on variables associated with week 12 treatment response and the three apoE isoforms. Metabolic and viral variables that showed non-parametric distributions were either $\log_{10}$-transformed to parametric distributions before hypothesis tests were applied or non-parametric tests (Kruskal–Wallis) were used and summarised as median (Q1–Q3). Univariate analysis of association was performed using Pearson correlation to test associations between parametric variables and Spearman's Rho for association between non-parametric variables. The regression (r) coefficients and a probability (p) from testing against the null hypothesis of no association were determined. p-Values <0.05 were considered to be significant. Variables with significant univariate correlations were analysed for evidence of independent association by multivariate linear regression analysis. Variables associated with week 12 treatment outcome were analysed using a binary logistic regression model. All statistical analyses were performed using Minitab16 (Minitab Inc., State College, USA).

Results

Patient characteristics

Seventy-two predominantly Caucasian patients were recruited to the study. Twenty-seven patients were treatment naïve at the

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Table 1. Patients characteristics.

	Overall cohort	Week 12 interferon response			
Treatment status	n = 72	Complete EVR n = 12	Partial EVR n = 20	Null n = 29	*p* value ANOVA
Naive	n = 27	9	5	2	
Experienced	n = 45	3	15	27	
Not treated	n = 11				
Age (yr)	48.6 ± 9.7	43.3 ± 11.7	48.6 ± 9.1	50.9 ± 7.4	0.055
BMI (kg/m^2)	25.4 ± 4.0	24.3 ± 3.4	25.0 ± 4.3	27.2 ± 3.4	0.036
Liver stiffness (kPa)[#]	7.3 (5.3-16.8)	5.5 (4.3-7.3)	7.9 (5.0-27)	10.2 (6-29.7)	0.016*
apoB (g/L)	0.88 ± 0.25	1.0 ± 0.2	0.84 ± 0.04	0.86 ± 0.29	0.144
apoE (mg/L)	33 ± 14	23.9 ± 7.7	35.0 ± 13.2	36.1 ± 15.3	0.068
apoA-I (g/L)	1.47 ± 0.29	1.42 ± 0.34	1.44 ± 0.18	1.43 ± 0.30	0.984
LDL cholesterol (mmol/L)	2.8 ± 0.9	3.27 ± 0.73	2.43 ± 0.78	2.64 ± 0.90	0.039
Triglycerides (mmol/L)[#]	1.16 (0.9-1.5)	1.2 (0.3-1.5)	1.0 (0.8-1.2)	1.3 (1.1-1.9)	0.085*
HOMA IR[#]	1.39 (0.81-2.2)	0.92 (0.32-1.48)	1.24 (0.67-2.18)	1.86 (1.11-2.45)	0.160
IP10 (pg/ml)[#]	279 (169-553)	258 (176-313)	273 (178-428)	543 (163-708)	0.052*
LVP (log_{10} IU/ml)	5.21 ± 0.67	5.20 ± 0.69	5.29 ± 0.70	5.18 ± 0.67	0.889
Non-LVP (log_{10} IU/ml)	5.78 ± 0.62	5.97 ± 0.73	5.84 ± 0.63	5.59 ± 0.61	0.189
Total viral load (log_{10} IU/ml)	5.93 ± 0.57	6.05 ± 0.70	6.06 ± 0.56	5.87 ± 0.56	0.794
LVP ratio	0.27 ± 0.19	0.16 ± 0.07	0.26 ± 0.15	0.32 ± 0.20	0.022
IL28B CC frequency	18/51 (35%)	5/8 (63%)	7/15 (47%)	4/22 (18%)	0.044[##]

Data are mean ± SD except where indicated.
*One way ANOVA performed on log_{10} triglycerides, log_{10} IP10, 1/fibroscan, log_{10} HDL.
[#]Median (Q1–Q3).
[##]Chi Square test.

time of the study, of whom 16 have subsequently been treated and 11 remained untreated. Of those treated, 9/16 (56%) achieved a complete EVR with subsequent SVR, 5/16 (31%) were relapsers after a partial EVR and 2/16 (13%) were null responders. Forty-five patients were treatment experienced, of whom 27/45 (60%) were prior null responders and 18/45 (40%) were prior relapsers.

Table 1 indicates variation in clinical and metabolic characteristics in the overall cohort and between complete EVR, partial EVR and null responders. Null responders had a clinical phenotype of older age, higher BMI, higher liver stiffness measurements (LSM), lower LDL cholesterol and apoB, and higher apoE levels than complete EVRs. The overall frequency of the major C allele of rs12979860 was 0.61 (CC = 35.3%, CT = 51%, TT = 13.7%), and the CC genotype was more prevalent in complete EVRs than null responders (63% *vs.* 18%). There was no significant variation in lipid profiles between visits in those 27 patients who attended for a second study visit (Supplementary Table 1).

IP10 correlates with apoB and liver stiffness

IP10 was measured as marker of ISG expression. The average intra-assay CV for the IP10 ELISA was 3.7% and inter-assay CV was 6.1%. IP10 levels were wide ranging (median 279; min 65, max 1642 pg/ml) and often high, consistent with significant induction of ISGs (Table 1). IP10 levels tended to be higher in prior null responders (Table 1). IP10 levels did not correlate directly with any specific viral parameter (*vs.* total viral load r = −0.021, $p = 0.864$; *vs.* LVP r = 0.179, $p = 0.150$; *vs.* non-LVP r = 0.035, $p = 0.782$; *vs.* LVP ratio r = 0.167, $p = 0.166$).

A relationship between ISGs and lipids was evaluated by correlation analysis of serum IP10 with lipid profiles. There was a significant positive correlation between IP10 and apoE (r = 0.369, $p = 0.004$). There was also a significant negative correlation between IP10 and total cholesterol (r = −0.314, $p = 0.009$). This relationship was restricted to LDL-C (r = −0.481, p <0.001) rather than HDL-C (r = 0.060, $p = 0.657$) and was likewise reflected by a negative correlation of IP10 with apoB (r = −0.446, $p < 0.001$) (Table 2). The negative correlation between IP10 and LDL-C and apoB was confirmed independently in 27 patients attending for a second visit (IP10 *vs.* LDL-C, r = −0.498, $p = 0.026$; IP10 *vs.* apoB, r = −0.464, $p = 0.034$) although the positive correlation between IP10 and apoE was no longer significant in these smaller numbers (r = 0.351, $p = 0.109$). IP10 also significantly correlated with liver stiffness (r = 0.390, $p = 0.002$). In multivariate linear regression analysis, liver stiffness ($p = 0.032$) and the negative correlation with apoB ($p = 0.001$) were the only independent predictors of IP10 levels ($R^2 = 27.7\%$).

ApoE correlates with HCV–LVP

The mean apoE level was 33 ± 14 mg/L and ranged from 12 to 71 mg/L for HCV-G1 patients (Table 1). ApoE levels correlated with fasting triglycerides (r = 0.467, $p = 0.001$) and trended towards variation corresponding to apoE genotype similarly to previous reports [38] with increased apoE levels in those with

Table 2. Viral and metabolic correlations.

	Univariate analysis			Multivariate linear regression, controlling for *IL28B*		
Outcome variable	Explanatory variable	Correlation coefficient r		Predictor	*p* value	R^2 (%)
		r	*p*			
LVP (log_{10} IU/ml)	apoE	0.283	0.034	apoE	0.003	19.5
LVP ratio	apoE	0.313	0.014	apoE	0.042	17.1
	HOMA IR	0.280	0.019	HOMA IR	0.028	
	Liver stiffness	0.253	0.042#			
apoE (mg/L)	LDL-C	-0.452	0.001	LDL-C	<0.001	50.6
	LVP	0.283	0.034	Triglycerides*	0.002	
	LVP ratio	0.313	0.014	LVP	0.017	
	IP10	0.369	0.004#			
	Triglycerides	0.467	0.001			
IP10 (pg/ml)	apoE	0.369	0.004	apoB	0.001	27.7
	apoB	-0.446	<0.001	Liver stiffness*	0.032	
	LDL-C	-0.481	<0.001			
	Liver stiffness	0.390	0.002#			
LDL cholesterol (mmol/L)	apoB	0.888	<0.001	apoB	<0.001	80
	apoE	-0.452	0.001	apoE	0.002	
	IP10	-0.462	<0.001			
	Liver stiffness	-0.425	0.001#			
apoB (g/L)	IP10	-0.446	<0.001	LDL-C	<0.001	90
	LDL-C	0.888	<0.001	Triglycerides*	<0.001	
	Triglycerides	0.472	<0.001			
	Liver stiffness	-0.309	0.013			
Triglycerides (mmol/L)	BMI	0.265	0.049	apoB	<0.001	47.2
	HOMA IR	0.320	0.016	apoE	<0.001	
	apoB	0.472	<0.001			
	apoE	0.467	0.001			
Liver stiffness# (kPa)	apoB	-0.309	0.013#	IP10*	0.003	22.3
	IP10	0.390	0.002#	HOMA IR	0.014	
	LVP ratio	0.253	0.042#			
	HOMA IR	0.320	0.009#			
HOMA IR	Liver stiffness	0.320	0.009#	Liver stiffness*	0.045	30.7
	LVP ratio	0.280	0.019	LVP ratio	0.015	
	BMI	0.347	0.003	BMI	<0.001	
	Triglycerides	0.320	0.016			

Pearson correlation was used except where indicated.

Spearman's Rank correlation performed on non-parametric data for univariate analysis.

* Transformed to log_{10} triglycerides, log_{10} IP10, and 1/Liver stiffness for multivariate linear regression analysis. Non-LVP and total viral load had no significant metabolic correlates.

E2/E3 *vs.* E3/E3 or E3/E4 (Supplementary Table 2). Serum apoE levels tended to be lower in those who achieved complete EVR ($p = 0.068$) (Table 1). In univariate analysis, serum levels of apoE correlated significantly with LVP load ($r = 0.283$, $p = 0.034$) and LVP ratio ($r = 0.313$, $p = 0.014$) (Fig. 1 and Table 2). The correlation of apoE with LVP and LVP ratio was confirmed independently in the second fasting sample provided by 27 individuals (apoE *vs.* LVP-load $r = 0.553$, $p = 0.040$; apoE *vs.* LVP ratio $r = 0.555$, $p = 0.041$). There was no significant correlation between apoE and total viral load ($r = -0.075$, $p = 0.567$) or apoE and non-LVP ($r = 0.011$, $p = 0.937$). This was confirmed in the second sample (apoE *vs.* total viral load $r = 0.147$, $p = 0.560$; apoE *vs.* non-LVP $r = -0.177$, $p = 0.512$). ApoE also correlated with IP10 ($r = 0.369$, $p = 0.004$) and triglycerides ($r = 0.467$, $p = 0.001$), and negatively with LDL-C ($r = -0.452$, $p = 0.001$). In multivariate linear regression analysis, apoE remained significantly associated independently with LDL-C ($p < 0.001$), triglycerides ($p = 0.002$), and LVP ($p = 0.017$) ($R^2 = 50.6\%$, Table 2).

LVP ratio is associated with treatment outcome

LVP ratio was higher in null responders than complete or partial EVRs ($p = 0.022$) (Table 1). EVR was also associated with higher LDL-C ($p = 0.039$), lower BMI ($p = 0.036$), lower liver stiffness ($p = 0.016$) and *IL28B* CC genotype ($p = 0.044$). Binary logistic regression analysis of these factors associated with EVR (partial or complete) *vs.* null response revealed that LVP ratio was the most significant independent predictor of EVR (odds ratio 0.01, 95% CI 0.00–0.042, $p = 0.018$) (Table 3), indicating that those with high LVP ratios were less likely to achieve EVR.

Variation of lipid profiles with IL28B genotype

Serum apoE levels were significantly lower in the response-favourable rs12979860 CC genotype compared to the unfavourable CT/TT genotype (28 mg/L ± 11 *vs.* 35 mg/L ± 13, $p = 0.048$)

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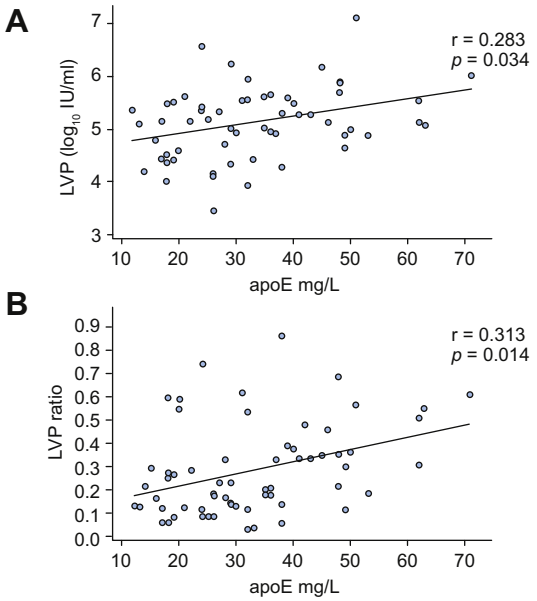

Fig. 1. Correlation between HCV lipoviral particles and apolipoprotein E (apoE). Plasma from 72 HCV genotype-1 patients in the fasting state was fractionated using 12.5% iodixanol density gradient ultracentrifugation. Lipoviral particles (LVP) were defined from HCV RNA detected in the low density fraction (d <1.07 g/ml). LVP ratio was calculated (LVP ratio = LVP/LVP + non-LVP). Serum apoE correlated significantly with (A) LVP log_{10} IU/ml (r = 0.283, $p = 0.034$) and (B) LVP ratio (r = 0.313, $p = 0.014$) (Pearson correlation analysis).

Table 3. Binary logistic regression analysis of factors associated with EVR (partial or complete *vs.* null).

Factor	Odds ratio	95% CI	*p* value
LVP ratio	0.01	0.00-0.42	0.018
BMI	0.83	0.69-1.01	0.058
LDL-C	1.37	0.60-3.12	0.452
Liver stiffness	1.01	0.96-1.07	0.612

(Table 4). CT/TT genotype was associated with significantly lower LDL-C (2.46 ± 0.82 mmol/L *vs.* 3.19 ± 0.75 mmol/L, $p = 0.006$) and lower apoB (0.8 ± 0.2 *vs.* 1.0 ± 0.2 g/L, $p = 0.005$). Triglycerides, HDL-C and apoA-I were not significantly different between *IL28B* genotypes.

Variation of viral parameters with IL28B and apoE genotype

We sought to evaluate whether rs12979860 was associated with variation in LVP. Total plasma HCV RNA, LVP-load, non-LVP load and LVP ratio were compared between CC and CT/TT genotypes. HCV RNA total viral load was significantly higher in those with response-favourable CC genotype (6.11 ± 0.63 log_{10} IU/ml *vs.* 5.72 ± 0.49, $p = 0.022$). This difference in total viral load was predominantly accounted for by a higher non-LVP fraction in CC genotype (6.09 ± 0.58 log_{10} IU/ml *vs.* 5.59 ± 0.62 log_{10} IU/ml, $p = 0.008$) and reflected by a trend towards lower LVP ratio (CC median 0.15 (0.11–0.26 Q1–Q3) *vs.* CT/TT median 0.26 (0.13–0.36 Q1–Q3), $p = 0.190$). There was no significant difference in LVP-load between the *IL28B* genotypes.

Table 4. Comparison of HCV viral load, lipoviral particles, IP10 and lipid profiles between *IL28B* rs12979860 CC and CT/TT genotypes.

rs12979860	CC (favorable) n = 18	CT/TT n = 33	*p* value
Total viral load (log_{10} IU/ml)	6.11 ± 0.63	5.72 ± 0.49	0.022
LVP (log_{10} IU/ml)	5.42 ± 0.78	5.10 ± 0.67	0.124
Non-LVP (log_{10} IU/ml)	6.09 ± 0.58	5.59 ± 0.62	0.008
LVP ratio[#]	0.15 (0.11-0.26)	0.26 (0.13-0.36)	0.190
IP10 (pg/ml)[#]	262 (172-381)	408 (174-682)	0.163
LDL cholesterol (mmol/L)	3.19 ± 0.75	2.46 ± 0.82	0.006
Triglycerides (mmol/L)[#]	1.15 (1.00-1.50)	1.13 (0.90-1.51)	0.558
apoB (g/L)	1.0 ± 0.2	0.8 ± 0.2	0.005
apoE (mg/L)	28 ± 11	35 ± 13	0.048
apoA-I (g/L)	1.42 ± 0.31	1.47 ± 0.27	0.610

Mean ± SD, 2-sample *t* test.
[#] Median (Q1–Q3) Kruskal–Wallis test.

We evaluated variation in LVP with apoE genotype because of correlation of LVP and LVP ratio with apoE. Frequencies of the apoE isoforms were E2 = 0.08, E3 = 0.73, and E4 = 0.19. No significant differences in these viral parameters were identified between apoE genotypes (Supplementary Table 2).

Discussion

The present study evaluated the hypothesis that levels of infectious LVP *in vivo* are associated with interferon sensitivity. The clinical phenotype of null response to peginterferon-α and RBV in our population was characterised by increased LVP ratios. In contrast, EVR was associated with low LVP ratio, low apoE and increased apoB and LDL-C. Low apoE levels were also associated with the favourable *IL28B* CC genotype. ApoE correlated with LVP and LVP ratio and was the strongest predictor of LVP in multivariate linear regression analysis.

It is established that LVP are low-density HCV particles that have high infectivity [1,6]. LVP may mask neutralising epitopes, thus evading recognition by neutralising antibodies [39,40]. We have previously reported that LVP correlate with insulin resistance and triglycerides in HCV-G1 infection [7]. High apoE levels are likely to be advantageous to the HCV lifecycle. ApoE is enriched on the surface of LVP [15–17] and is important for cell entry [18]. Viral non-structural proteins including NS5A interact with several host proteins involved in regulation of lipid metabolism at the sites of viral replication [41,42]. An interaction between NS5A and apoE is required for formation of infectious virus [2,12,14,43]. High levels of apoE may therefore be advantageous for the HCV-G1 lifecycle and favour the formation of infectious LVP *in vivo*.

We were unable to determine whether LVP reduces interferon sensitivity directly, or whether the effects of the interferon response on lipids may impact indirectly on levels of LVP.

The data in this study suggest a direct effect of ISGs on lipid profiles. The notion that interferons can alter lipids was recogni-

sed by reports of increased serum triglycerides in the early studies of IFNα therapy [44]. The endogenous interferon response may also alter lipid metabolism. IP10 is an immune-modulatory chemokine and has been considered a candidate serum marker for ISG expression [45]. High ISGs are associated with non-response to peginterferon-α treatment and with the unfavourable *IL28B* genotype [46,47]. Serum IP10 levels have been reported to be predictive of viral kinetics [31,45,48,49] and non-response to Peg-IFNα and RBV [30,50,51]. However, IP10 as a single serum marker is unlikely to represent a complete measure of hepatic ISG activation. Notably, unlike ISG expression in the liver, IP10 levels did not significantly differ by *IL28B* genotype, although we observed increased IP10 levels in null responders. Univariate analysis demonstrated a positive correlation between IP10 and apoE, and a negative correlation with LDL-C and apoB. Multivariate linear regression demonstrated that high IP10 levels were associated with increased liver stiffness and low apoB, supporting the concept that endogenous ISG activation alters VLDL/LDL metabolism and may therefore indirectly favour formation of LVP.

ISGs are also regulated by *IL28B* genotype. Our new data indicate that the unfavourable CT/TT genotype of rs12979860 was associated with increased levels of apoE but decreased levels of LDL-C and apoB. These data are consistent with a previous report of lipid profiles in HCV-G1 that indicated higher LDL-C and apoB levels in those with the favourable CC genotypes than those with CT/TT [36]. The influence of *IL28B* genotype on lipid profiles in the general population has not been reported. We hypothesise that a state of high endogenous interferon activation also causes altered lipid profiles, characterised by low apoB and LDL-C but increased apoE, and insensitivity to treatment with peginterferon-α. Low apoB and LDL-C may therefore be surrogate markers that reflect ISG activation and may help explain the clinical associations of low LDL-C with non-response to anti-viral therapy [32–34].

An alternative hypothesis is that HCV infection *per se* alters lipid levels and subsequently modifies ISGs expression and interferon sensitivity. Experimental evidence indicates that LVP from chronically infected HCV patients can interfere with maturation of monocyte-derived dendritic cells induced by TLR3 or TLR4 ligands, supporting the hypothesis that LVP may interfere with interferon sensitivity [52].

It remains a paradox that the favourable *IL28B* genotype is associated with higher total viral load, given the association of low viral load with sustained virological response, but this has been a consistent and hitherto unexplained association with *IL28B* genotype [21]. Our data indicate that this difference in viral load between *IL28B* genotypes is manifested largely in the high density 'non-LVP' fraction that has lower infectivity *in vitro*. Further studies in larger cohorts are required to evaluate whether the association of LVP and non-response to anti-viral treatment [7] are independent of *IL28B* genotype. However, these preliminary data suggest some variation may be explained by the relative increase of higher density (d >1.07 g/ml)/lower infectivity 'non-LVP' in those with the favourable *IL28B* genotype. In contrast, elevated apoE levels in those with unfavourable *IL28B* genotype may promote production of more infectious LVPs *in vivo*.

In summary, our data indicate that apoE is a key mediator in a complex interaction between interferon sensitivity, lipoprotein metabolism and infectious virus in chronic HCV infection. High apoE levels were associated with increased levels of LVP, and null response to peginterferon-α. ApoE was significantly higher in those with the unfavourable *IL28B* genotype. In contrast, low apoE correlated with low LVP, increased LDL-C, the favourable CC *IL28B* genotype and EVR. Further understanding of the effects of innate antiviral responses on lipid pathways in HCV may therefore provide novel therapeutic targets to enhance or restore interferon sensitivity, which may be a critical factor in preventing resistance to DAAs [29].

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Conflicts of interest

The authors of this study declare that they do not have anything to disclose regarding funding or conflicts of interest with respect to this manuscript.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2012.02.017.

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