Research Article

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Apolipoprotein H expression is associated with *IL28B* genotype and viral clearance in hepatitis C virus infection

Melissa E. Laird[1,2], Amira Mohsen[3], Darragh Duffy[1,2], Rasha Mamdouh[4], Lenaig LeFouler[5], Armanda Casrouge[1,2], Mai El-Daly[6], Mona Rafik[4], Mohamed Abdel-Hamid[6,7], Alexandre Soulier[8,9], Jean-Michel Pawlotsky[8,9], Christophe Hézode[9,10], Isabelle Rosa[9,10], Philippe Renard[11], Mostafa K. Mohamed[6], Philippe Bonnard[12,13], Jacques Izopet[14,15], Vincent Mallet[16,17,18], Stanislas Pol[16,17,18], Matthew L. Albert[1,2,18,*,†], Arnaud Fontanet[5,19,*,†]

[1]*Laboratory of Dendritic Cell Immunobiology, Institut Pasteur, Paris, France;* [2]*INSERM U818, Paris, France;* [3]*Community Medicine Department, National Research Center, Cairo, Egypt;* [4]*Faculty of Medicine, Ain Shams University, Cairo, Egypt;* [5]*Emerging Disease Epidemiology Unit, Institut Pasteur, Paris, France;* [6]*Liver Disease Research Unit, National Hepatology & Tropical Medicine Research Institute, Cairo, Egypt;* [7]*Faculty of Medicine, Minia University, Egypt;* [8]*National Reference Center for Viral Hepatitis B, C, and D, Department of Virology, Hôpital Henri Mondor, Université Paris-Est, Créteil, France;* [9]*INSERM U955, Créteil, France;* [10]*Department of Hepatology and Gastroenterology, Hôpital Henri Mondor, Université Paris-Est, Créteil, France;* [11]*Department of Gastroenterology and Hepatology, Hôpital Victor Dupouy, Argenteuil, France;* [12]*Maladies Infectieuses et Tropicales, Hôpital Tenon (APHP), Paris, France;* [13]*INSERM U-707, UPMC, Paris, France;* [14]*Department of Virology, CHU Toulouse, Toulouse, France;* [15]*INSERM U1043, IFR-BMT, Toulouse, France;* [16]*Université Paris Descartes, Paris, France;* [17]*Institut Cochin, INSERM (IMR-S1016), CNRS (UMR 8104), Paris, France;* [18]*Assistance Publique – Hôpitaux de Paris (APHP), Groupe Hospitalier Cochin Saint-Vincent de Paul, Unité d'Hepatologie, Paris, France;* [19]*Conservatoire National des Arts et Métiers, Paris, France*

Background & Aims: HCV requires host lipid metabolism for replication, and apolipoproteins have been implicated in the response to treatment.

Methods: We examined plasma apolipoprotein concentrations in three cohorts of patients: mono-infected patients with symptomatic acute hepatitis C (aHCV); those undergoing treatment for chronic hepatitis C (cHCV); and HIV/HCV co-infected patients being treated for their chronic hepatitis C. We also evaluated associations between apolipoproteins and *IL28B* polymorphisms, a defined genetic determinant of viral clearance.

Results: Plasma apolipoprotein H (ApoH) levels were significantly higher in patients who achieved spontaneous clearance or responded to pegylated-interferon/ribavirin therapy. Strikingly, patients carrying the *IL28B* rs12979860 CC SNP correlated with the plasma concentration of ApoH in all three cohorts. Both ApoH and *IL28B* CC SNP were associated with HCV clearance in univariate analysis. Additional multivariate analysis revealed that the association between *IL28B* and HCV clearance was closely linked to that of Apo H and HCV clearance, suggesting that both belong to the same biological pathway to clearance. The association between *IL28B* CC SNP and ApoH was not observed in healthy individuals, suggesting that early post-infection events trigger differential ApoH expression in an *IL28B* allele dependent manner.

Conclusions: This relationship identifies ApoH as the first induced protein quantitative trait associated with *IL28B*, and characterises a novel host factor implicated in HCV clearance.

Keywords: Hepatitis C virus; Quantitative trait loci; Lipid metabolism; Apolipoproteins; *IL28B*.

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* Corresponding authors. Addresses: Unit of Dendritic Cell Immunobiology, Institut Pasteur & Inserm U818, Paris, France. Tel.: +33 1 45 68 89 02; fax: +33 1 45 68 85 48 (M.L. Albert). Unit of Emerging Disease Epidemiology, Institut Pasteur, Paris, France. Tel.: +33 1 40 61 37 66; fax: +33 45 68 88 76 (A. Fontanet).
E-mail addresses: albertm@pasteur.fr (M.L. Albert), fontanet@pasteur.fr (A. Fontanet).

† These authors share senior authorship.

Abbreviations: HCV, Hepatitis C virus; VLDL, very low density lipoprotein; Apo, apolipoprotein; aHCV, acute hepatitis C; cHCV, chronic hepatitis C; LVP, lipoviral particle; ALT, serum alanine aminotransferase; RNA, ribonucleic acid; PCR, polymerase chain reaction; MAP, multi-analyte profiling; SNP, single nucleotide polymorphism; IFNλ, interferon lambda; CL, cleared patient; NCL, not cleared; EVR, early virologic response; SVR, sustained virologic response; NR, non-responder; HIV, human immunodeficiency virus.

Introduction

Hepatitis C virus (HCV) represents a serious public health problem, infecting approximately 170 million people worldwide [1]. Around 70% of infected individuals will progress to chronic HCV infection, one third of which are at significantly increased risk of progressive liver fibrosis, cirrhosis, and hepatocellular carcinoma. Recent advances in the deployment of first generation direct acting antivirals (DAAs), and the development of second generation DAAs, have been major steps forward in the eradication of HCV infection [2–5].

HCV is known to be critically dependent on host lipid metabolism. HCV proteins require intracellular association with lipid droplets for productive virion assembly [6], and circulating

infectious virions associate with very low-density lipoprotein (VLDL)-like particles, referred to as lipoviral particles (LVP) [7]. Incorporation of host apolipoproteins (apos) into the LVP is postulated to assist in viral entry [7–9], and several apolipoproteins are necessary for viral assembly and the production of infectious virions [10–12]. Additionally, elevated lipid levels have been associated with the response to pegylated interferon α_2/ribavirin (P/R) therapy during chronic HCV infection, suggesting a role for host proteins in viral clearance [13,14].

Genome wide association studies (GWAS) have identified a striking association between single nucleotide polymorphisms (SNPs) near the *IL28B* locus and spontaneous clearance of untreated HCV infections, as well as response to PR therapy [15–18]. For the rs12979860 SNP within the *IL28B* locus, the "protective" CC genotype confers a 2- to 3-fold higher rate of spontaneous viral clearance or sustained virologic response (SVR) following PR treatment, as compared to either the CT or TT haplotype [16–18]. Recently, a dinucleotide SNP in strong linkage disequilibrium with the rs12979860 SNP was shown to determine expression of a newly discovered gene product, IFN-λ_4 [19,20]. In addition, higher plasma levels of ApoB have been reported to be associated with a sustained virological response in patients carrying the rs8099917 responder genotype (located proximal to rs12979860) in IL28B [21].

In this study, we report ApoH as a correlate of both spontaneous clearance and response to therapy in HCV patients. Moreover, we observed that the ApoH concentration was higher in subjects carrying the CC allele of the *IL28B* haplotype. This was confirmed across three independent patient cohorts infected with two different HCV genotypes (1 and 4). Based on multivariate analysis, we show that the association between *IL28B* and HCV clearance is closely linked to that of ApoH and HCV clearance, suggesting that both belong to the same biological pathway to clearance. These findings open new areas for HCV research and may prove critical to defining the mechanism behind the strong genetic association between *IL28B* and HCV clearance.

Patients and methods

Patient, cohort and study group information

Clinical study details, patient information, sample collection and monitoring of the three HCV cohorts are described in Supplementary Table 1.

Multi-analyte profiling

Plasma was collected from fasting patients in Vacutainer blood collection tubes containing Sodium Heparin (BD) and stored at −80 °C. Plasma was cleaned by high-speed centrifugation and analysed using the Luminex xMAP technology. The measurement of 11 apolipoproteins was performed by Myriad Rules-Based-Medicine, (Austin, TX), according to guidelines set by the USA Clinical and Laboratory Standards Institute.

IL28B genotype determination

IL28B genotype at SNP position rs12979860 was determined by real-time PCR using genomic DNA extracted from frozen serum samples in conjunction with minor groove binder probes, [22] and as described in Supplementary materials and methods.

Statistical analyses

Categorical and continuous data were compared across groups using χ^2 and Mann-Whitney tests, respectively and as indicated. Mixed linear regression models were used to analyse longitudinal apolipoprotein expression data according to spontaneous clearance status in the acute hepatitis C cohort.

Logistic regression models were used to estimate the increase in the odds of clearance associated with *IL28B* variants (CC *vs.* CT-TT combined) and ApoH plasma concentrations (introduced as a categorical variable, in quartiles, in the model). Models were built for each of the three cohorts. Variables (*IL28B* SNP and ApoH) were tested in separate models first to estimate their individual effect on clearance, and then introduced simultaneously in the same model to estimate their independent effect on clearance. Since the HCV chronic cohort included both naïve and treated patients, odds-ratios were adjusted for history of treatment. For the model using HIV/HCV co-infected patients, odds-ratios for *IL28B* variants were calculated using median unbiased estimates with exact logistic regression models as all (9/9) *IL28B* CC variants cleared the virus under treatment (this would translate into an infinitely positive odds-ratio in classical logistic regression models).

Results

To address the potential impact of the rs12979860 *IL28B* SNP on host apolipoprotein levels and HCV clearance, the levels of plasma apolipoproteins and *IL28B* SNP genotypes were evaluated in three different HCV cohorts: (i) acute HCV (aHCV) patients in Egypt (n = 33), (ii) chronic HCV (cHCV) patients receiving standard P/R treatment (n = 141), and (iii) HIV/HCV co-infected patients treated with a double dose of PegIFN and a standard dose of RBV (n = 43). Summarised clinical characteristics of each cohort are provided in Supplementary Table 1.

The high incidence of HCV genotype 4 in Egypt (14.7% seropositivity) provides the unique opportunity to identify acute, symptomatic HCV patients, and potentially factors that influence spontaneous viral clearance [23–25]. Patients were recruited after presenting with symptoms to two hospitals in Cairo, as described in the Supplementary data, and followed for at least six months after the onset of symptoms. Of the 33 aHCV patients examined, 19 spontaneously cleared their virus ("cleared", CL), and the remaining 14 individuals developed chronic HCV infection ("not cleared", NCL). We examined 11 apolipoproteins for differences between CL and NCL groups, and for associations with the *IL28B* SNP (Supplementary Table 2). ApoCI was associated with the *IL28B* SNP, but did not correlate with viral clearance. Only ApoH demonstrated significant associations with both HCV clearance ($p = 0.005$, Fig. 1A) and the CC *IL28B* allele. ApoH

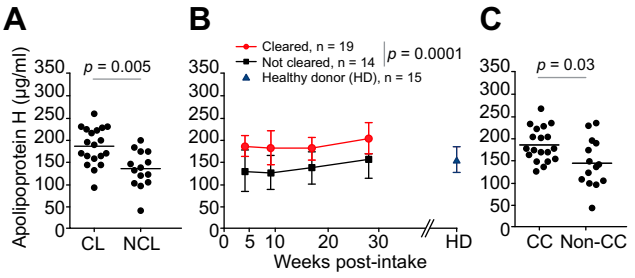

Fig. 1. Plasma ApoH is strongly associated with *IL28B* genotype and spontaneous viral clearance in acute HCV infection. (A) Plasma ApoH in patients that spontaneously cleared (CL, n = 19) or failed to clear virus (NCL, n = 14); Mann-Whitney $p = 0.005$. (B) Kinetics of plasma ApoH in CL (●) and NCL patients (■), mean values and standard deviation are shown. Mixed model longitudinal regression analysis was performed ($p < 0.0001$). (C) Associations between *IL28B* rs12979860 polymorphisms and apoH were examined. Elevated plasma ApoH is observed in CC patients (n = 19), compared to those with the CT or TT SNP ("non-CC") (n = 14); M-W $p = 0.03$. (This figure appears in colour on the web.)