Published in final edited form as:

Hepatology. 2010 June ; 51(6): 1904–1911. doi:10.1002/hep.23592.

Interferon lambda genotype and low serum LDL cholesterol levels in patients with chronic hepatitis C infection

Josephine H. Li¹, Xiang Qian Lao, MD, PhD¹, Hans L. Tillmann, MD², Jennifer Rowell, MD³, Keyur Patel, MD², Alexander Thompson, MD, PhD², Sunil Suchindran¹, Andrew J. Muir, MD², John R. Guyton, MD³, Stephen D. Gardner, MSPH⁴, John G. McHutchison, MD², and Jeanette J. McCarthy, MPH, PhD¹

¹Institute for Genome Sciences and Policy, Duke University Medical Center, Durham, NC

²Duke Clinical Research Institute and the Division of Gastroenterology, Duke University Medical Center, Durham, NC

³Division of Endocrinology, Duke University Medical Center, Durham, NC

⁴GlaxoSmithKline Research and Development, Research Triangle Park, NC

Abstract

Background—Recently, genetic polymorphisms occurring in the interferon lambda gene region were associated with response to interferon-based treatment of hepatitis C infection. Both infection with the hepatitis C virus and interferon therapy are associated with decreased serum cholesterol and high cholesterol has been associated with increased likelihood to respond to interferon. We sought to determine if the interferon lambda gene variant was also associated with serum lipid levels in chronic hepatitis C patients. We compared genotypes of the rs12979860 polymorphism, located proximal to the *IL28* gene, with serum lipid and apolipoprotein levels in 746 subjects with chronic HCV infection, not currently undergoing treatment, using multivariable analysis of variance.

Results—Levels of total cholesterol ($p=6.0 \times 10^{-4}$), apolipoprotein B ($p=1.3 \times 10^{-6}$) and low density lipoprotein (LDL) cholesterol ($p=8.9 \times 10^{-10}$) were significantly higher in subjects carrying the rs12979860 CC ‘responder’ genotype compared to those with the CT or TT genotype. Levels of triglycerides ($p=0.03$), apolipoprotein A-I ($p=0.06$) and apolipoprotein E ($p=0.01$) were slightly lower in the rs12979860 CC genotype group, while levels of high density lipoprotein cholesterol ($p=0.78$) and apolipoprotein C-III ($p=0.74$) did not vary by rs12979860 genotype.

Conclusions—Our results suggest that low levels of LDL cholesterol in chronic hepatitis C patients may be a marker of host endogenous interferon response to hepatitis C and that subjects with the rs12979860 CC ‘responder’ genotype may have a lower endogenous interferon response to the virus.

Keywords

genetic; lipids; IL28; treatment response

There is a growing body of epidemiological literature to support an association between Hepatitis C virus (HCV) infection and alterations in host serum lipid levels. A consistent, strong association exists between chronic hepatitis C (CHC) infection and low levels of total

cholesterol, low density lipoprotein (LDL) cholesterol and apolipoprotein B, the main protein constituent of LDL and very low density lipoprotein (VLDL) (1-10). Moreover, there may be clinical implications for HCV-associated dyslipidemia. Low lipid levels in HCV infection have been correlated with steatosis(11,12), more advanced liver fibrosis(11,13-15) and non-response to interferon treatment(16-23).

Recently, several highly correlated common single nucleotide polymorphisms on a linkage disequilibrium block encompassing two interferon lambda genes, encoding type III interferons $\lambda 2$ (*IL28A*) and $\lambda 3$ (*IL28B*) have been implicated in response to pegylated interferon α among CHC patients (24-26). CHC patients with the CC genotype of rs12979860 were more than twice as likely to respond to treatment than those without this genotype. In addition, this same genotype was paradoxically associated with higher serum HCV RNA levels. The identification of the causal genetic variant underlying this association and its function remain to be determined; however, given the genomic location of the associated SNPs, the causal variant is likely to influence levels or activity of one or both of these interferon lambdas. Treatment with Type I interferons has been shown to affect the metabolism of LDL(27,28), resulting in a marked decline in serum LDL cholesterol levels. Little is known about the effect of type III interferons on lipid metabolism. We sought to determine the association between the interferon lambda rs12979860 polymorphism and serum lipid levels in a cohort of patients with chronic HCV infection in order to further evaluate the inter-relationship of interferon lambda and HCV hypolipidemia.

Methods

Patient population

Subjects for the current study were drawn from two sources. Most came from the Duke Hepatology Clinical Research Database and Repository (29), which consists primarily of patients referred to the Duke Liver Clinic with a diagnosis of HCV infection. Some, but not all patients took part in clinical trials or received treatment as part of standard of care, while others had not received any treatment. At the time of enrollment, informed consent is obtained for the collection and storage of serum, liver tissue and peripheral blood for DNA extraction. The database includes clinical and demographic data extracted from medical records, laboratory reports and, for those patients enrolled in clinical trials, case-report forms. A second cohort of patients was from a recently completed multicenter Phase II clinical trial to assess the effectiveness of farglitazar, a PPAR γ agonist, as an antifibrotic agent among CHC patients who were non-responsive to prior interferon-based therapy (NCT 00244751). Samples and patient data collected at baseline were used in the current study and all patients gave consent for genetic studies.

HCV status was confirmed by presence of detectable serum HCV RNA and HCV genotypes determined by the INNOLIPA HCV assay (Innogenetics, Zwijnaarde, Belgium)(30). Liver fibrosis was scored using the METAVIR system (F0-F4). Patients who had undergone treatment with standard of care pegylated interferon/ribavirin (PEG-IFN/RBV), either as part of a clinical trial or regular care, were classified according to whether they had a sustained virologic response (SVR), defined as having undetectable HCV RNA levels 24 weeks after cessation of treatment, or non-SVR, defined as detectable HCV RNA levels at 24 weeks after cessation of treatment. HCV RNA levels in the Duke serum samples were measured using either the National Genetics Institute SuperQuant assay (Culver City, Los Angeles, CA) or the Cobas Taqman HCV Test (Roche Molecular Systems, Pleasanton, California) and for the farglitazar samples using Quantitative Real-Time PCR assay (Quest Diagnostics, Northridge, CA). Viral load was classified as low (<600,000 IU/ml) or high ($\geq 600,000$ IU/ml) for analysis. Body mass index (kg/m^2) was calculated from height and weight at enrollment.

All samples used for analysis of lipids were from subjects not currently undergoing treatment for HCV. Subjects with prior effective treatment, and thus no longer chronically infected with HCV, were not used in the current study. Frozen serum samples were analyzed at a National Heart, Lung, and Blood Institute/Centers for Disease Control and Prevention Lipid Standardization Program certified laboratory (N. Rifai, Children's Hospital, Boston, MA). Standard lipids (triglycerides, total-, HDL- and LDL-cholesterol) were measured directly with reagents from Roche Diagnostics (Indianapolis, IN). Apolipoprotein A-I, B, C-III, and E were measured with immunoturbidometric assays (DiaSorin, Stillwater, Minn). Samples from the Duke cohort were collected under non-fasting conditions, while those from the farglitazar study were collected under fasting conditions.

There were 634 subjects from the Duke cohort and 112 from the farglitazar study who had serum, DNA and clinical data available. We excluded subjects co-infected with hepatitis B virus or HIV-1; who had undergone liver transplantation; race that was other than Caucasian or African American; HCV genotypes that were not 1, 2 or 3; and the <1% of subjects with poor quality DNA, defined as >10% failed genotypes in prior test-genotyping across a minimum of 10 single nucleotide polymorphisms (SNPs). We excluded patients who had their lipids measured after attaining a SVR to treatment, as well as patients with unknown date of serum collection.

This study, the database and repository were approved by the Duke University Institutional Review Board. All patients provided written informed consent and the study was conducted in accordance with provisions of the Declaration of Helsinki and Good Clinical Practice guidelines.

Genotyping

The genomic region associated with HCV response in Ge *et al.*(24) contains several highly correlated SNPs around the *IL28B* gene. We selected the most strongly associated SNP, rs12979860, located upstream of this gene for genotyping in our cohort using the 5' nuclease assay with allele specific TaqMan probes(31). Genotyping was performed in the Duke Institute for Genome Sciences and Policy Genotyping Core. Genotyping calls were manually inspected and verified prior to release. Hardy-Weinberg Equilibrium was assessed in Caucasians and African Americans separately.

Statistical analysis

SAS statistical software, Version 9.1 (SAS Institutes, Cary, NC) was used to carry out linear regression analysis of rs12979860 genotypes associated with lipids. Rs12979860 genotypes were coded to test a recessive model, comparing subjects homozygous (CC) to those with one or no copies of the C allele (CT/TT). Triglyceride, apolipoprotein C-III, and apolipoprotein E levels were natural log (ln)-transformed prior to analysis. Primary analysis of the association between rs12979860 and lipids controlled for cohort type, age, sex, race, and HCV genotype. The amount of variance in lipid levels explained by rs12979860 was determined by the partial r^2 statistic. Homogeneity of genotype effects across strata was tested by introducing an interaction term. Differences in lipid levels by HCV genotype were examined using Analysis of Variance. To assess the effect of pre-treatment lipids compared to rs12979860 genotype as a predictor of response to PEG-IFN/RBV treatment, a multivariable logistic regression model was run controlling for age, sex, race, and fibrosis.

Results

Association of rs12979860 genotype with serum lipid levels

A description of the two CHC cohorts used in our analysis of serum lipid levels is shown in Table 1. Total cholesterol, HDL cholesterol and apolipoprotein E levels were measured on only a subset of subjects (n=577). Rs12979860 allele frequencies differed by race and were consistent with those previously reported (Caucasian C=.61, T=.39; African American C=.36, T=.64). Deviation from Hardy-Weinberg Equilibrium was only nominally significant ($p=0.05$) in Caucasians.

Results for the two cohorts were similar (Supplemental Table S1) and therefore, the cohorts were combined for further analysis. Table 2 illustrates the mean serum lipid levels of the entire cohort by rs12979860 genotype (CC vs. TT/TC), adjusting for cohort, age, sex, race, and HCV genotype. The most significant differences were for LDL cholesterol, apolipoprotein B and total cholesterol levels. Subjects with the rs12979860 TT/TC genotype had significantly lower LDL cholesterol levels ($p=8.9 \times 10^{-10}$), lower apolipoprotein B ($p=1.3 \times 10^{-6}$) and lower total cholesterol ($p=0.0006$) levels compared to subjects with the CC genotypes. Nominally significant associations between rs12979860 TT/TC and higher triglycerides, apolipoprotein E and apolipoprotein A-I were also found. Rs12979860 genotype was the strongest factor associated with LDL cholesterol and accounted for 5% of the variation in this trait in this cohort of patients with CHC. Additional models were run controlling for body mass index (BMI) and METAVIR fibrosis score on the subset of 566 subjects with these data available, and for viral load on the subset of 336 subjects with these data, but inclusion of these additional variables in the model did not alter the association (data not shown).

Association of serum lipids and rs12979860 genotype with HCV genotype

Next, we assessed for differences in serum lipid levels according to HCV genotype. Only total cholesterol, LDL cholesterol and apolipoprotein B levels varied significantly by HCV genotype: patients with HCV genotype 3 had the lowest total cholesterol, LDL cholesterol and apolipoprotein B levels, followed by genotype 1 and finally genotype 2 had the highest (Figure 1). However, rs12979860 genotype also varied by HCV genotype in our cohort (32), but the association between rs12979860 genotype and serum lipid levels did not vary significantly by HCV genotype (rs12979860 genotype by HCV genotype interaction $p=0.22$ for LDL cholesterol; $p=0.76$ for ApoB). As seen in Figure 2, a significant association between rs12979860 and both LDL cholesterol and apolipoprotein B levels was found among patients infected with HCV genotype 1. Patients with HCV genotypes 2 and 3 showed a similar trend, but the association failed to reach significance.

Effect of pre-treatment lipids levels on treatment response to PEG-IFN/RBV

Pre-treatment lipids were available on 261 chronic HCV patients who had received PEG-IFN/RBV treatment, 203 of whom had complete data on treatment response and other covariates. To avoid complex relationships introduced by HCV genotype, we utilized only the 182 HCV genotype 1 patients to determine the association between pre-treatment lipid levels and treatment response and whether an observed association was due to the effects of rs12979860 genotype. Characteristics of responders and non-responders are given in Supplemental Table S2. Both high levels of LDL cholesterol and rs12979860 CC genotype were associated with SVR to treatment when examined individually in their own multivariable models. However, inclusion of both of these predictors in the same multivariable model resulted in an attenuation of the effect of LDL cholesterol such that its association with treatment response was no longer significant, while the effect of rs12979860 genotype on treatment response remained highly significant (Table 3).

Discussion

The presence of low serum cholesterol levels in patients with CHC has been well-documented. In a large cohort of CHC patients, we now report for the first time that an interferon lambda gene variant recently associated with response to interferon therapy(24-26) was also strongly associated with serum lipoproteins. The rs12979860 CC 'responder' genotype was most significantly associated with higher levels of LDL cholesterol, apolipoprotein B, and total cholesterol. These associations reflect the same underlying relationship since most of total cholesterol is carried by LDL cholesterol and apolipoprotein B is the main apolipoprotein constituent of LDL cholesterol. These associations were independent of important covariates and may explain 5% of the variation in LDL cholesterol levels among chronic HCV patients. Furthermore, known proxies of the rs12979860 SNP are not genetic contributors to LDL cholesterol in healthy non-HCV-infected populations(33).

The rs12979860 polymorphism lies in a region between two interferon lambda genes, *IL28A* and *IL28B*, harboring multiple highly correlated gene variants. The causal genetic variant underlying the observed genetic associations at this locus is not yet known, but so far rs12979860 appears to be a strong predictor of HCV treatment response. The causal variant is likely to influence activity or levels of the nearby interferon lambda genes. The data are consistent with observations by others that responders to treatment are characterized as having a lower baseline immune response to HCV (34,35). This could also explain the paradoxical association of the 'response' genotype with higher viral load in the study Ge *et al.*(24) and in a follow-up report in the cohort used in the current study(32). Our data on rs12979860 genotype association with lipids support this theory as well. Administration of exogenous interferons alpha, beta, and gamma in the setting of treatment for chronic HCV infection and other conditions has been shown to lower LDL cholesterol, and raise triglyceride in VLDL, concomitant with suppression of lipoprotein lipase (28,36-38). The effect of interferon lambdas on serum lipids has not been previously explored, although it is believed that interferon lambdas stimulate an interferon signaling gene response via a common pathway with type 1 interferons(39). It is not unreasonable to expect that the effect of interferon on lipids extends to endogenous interferons as well. As such, we hypothesize that the interferon lambda rs12979860 CC 'responder' genotype, which was associated with both increased likelihood of treatment response and higher LDL cholesterol levels in our cohort, is associated with lower interferon lambda activity or lower intrahepatic interferon signaling gene expression.

Our study also confirmed the observation by others that higher pre-treatment levels of LDL cholesterol predict increased response to standard of care PEG-IFN/RBV in CHC patients (16-23). However, we note that this association may largely result from the confounding effects of interferon lambda, as inclusion of rs12979860 genotype in the model resulted in a substantial attenuation of the association. Hence, while LDL cholesterol levels may not provide any independent clinical utility beyond IL28 genotype for predicting treatment response, they may serve as a marker of endogenous interferon response to HCV infection.

Our analyses carefully controlled for many factors that may confound the observed associations. Nonetheless, there were several limitations to acknowledge. First, the research utilized an observational study design where serum lipid levels were measured from stored samples taken at a single point in time. The majority were non-fasting samples and as such, triglyceride levels may be increased from fasting levels. However, the direct measures of HDL cholesterol, LDL cholesterol and all of the apolipoproteins are not appreciably affected in the fed state(40). In any case, the misclassification bias associated with this measurement error would be non-differential with respect to rs12979860 genotype resulting in a

conservative estimate of association. Indeed, examination of the fasting samples by themselves yielded slightly stronger association (data not shown). Second, statins and other lipid lower medications are known to lower LDL cholesterol in healthy populations. We did not have accurate data on use of lipid lowering therapies in our cohort, but statins have been unfrequently used in HCV patients(41). Furthermore, medication use is unlikely to confound the association, since use of these therapies is unlikely to be dependent on a person's IL28 genotype. Another limitation of our study is the relatively small numbers of patients infected with HCV genotypes 2 and 3, which reduces the power to detect main effects among these groups or differences in effect between HCV genotypes.

The rs12979860 genotype explains only 5% of the variation in LDL cholesterol levels among HCV infected subjects in our study, but has no effect on LDL cholesterol levels among non-HCV infected subjects(33). While this is more than the variation explained by every gene polymorphism discovered to date in non-HCV infected subjects(33), it leaves much of the variation in HCV-associated LDL cholesterol unaccounted for. Just how much of the variation in LDL cholesterol levels is due to interferon response versus direct viral perturbation of the host lipid metabolism pathway versus other factors remains to be determined. The viral life cycle of HCV is tightly linked to host cholesterol metabolism(42) and experimental evidence suggests that HCV exploits hepatocyte cholesterol metabolism pathways for cellular entry, replication, assembly and secretion(43,44) (45,46) (47).

In conclusion, our research has shown an association between rs12979860 genotype and host serum lipid levels, suggesting a relationship between endogenous interferon response and lipids. Additional studies are warranted to assess the utility of serum lipids as a marker of host interferon response to HCV.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank all of the study participants who contributed their biospecimens and data to the Duke Hepatology Clinic Database and Biorepository and acknowledge Diane Uzarski, Crystal Cates, Chris Delionbach and Melissa Austin for continued maintenance of this valuable resource. We would also like to thank collaborators Dr. Arlene Hughes and Dr. Michael Mosteller at GlaxoSmithKline for facilitating the sharing of the farglitazar clinical trial data and providing valuable feedback on the manuscript. There are no other conflicts to disclose.

Financial Support: This study was funded in part by a generous grant from the David H Murdock Institute for Business and Culture via the M.U.R.D.O.C.K. Study and award 1 UL1 RR024128-01 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH) and NIH Roadmap for Medical Research, and its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH. Dr. Thompson received funding support from the Duke Clinical Research Institute, a generous research gift from the Richard B. Boebel Family Fund, the National Health and Medical Research Council of Australia and the Gastroenterology Society of Australia. Dr. Rowell is supported by NIH Training Grant T32-DK007012-31 at Duke University Medical Center.

References

1. Maggi G, Bottelli R, Gola D, Perricone G, Posca M, Zavaglia C, Ideo G. Serum cholesterol and chronic hepatitis C. *Ital J Gastroenterol.* 1996; 28:436–440. [PubMed: 9032585]
2. Fabris C, Federico E, Soardo G, Falletti E, Pirisi M. Blood lipids of patients with chronic hepatitis: differences related to viral etiology. *Clin Chim Acta.* 1997; 261:159–165. [PubMed: 9201435]
3. Moriya K, Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Yotsuyanagi H, Iino S, et al. Serum lipid profile of patients with genotype 1b hepatitis C viral infection in Japan. *Hepatol Res.* 2003; 25:371–376. [PubMed: 12699847]

4. Montes ML, Pulido F, Barros C, Condes E, Rubio R, Cepeda C, Dronda F, et al. Lipid disorders in antiretroviral-naïve patients treated with lopinavir/ritonavir-based HAART: frequency, characterization and risk factors. *J Antimicrob Chemother.* 2005; 55:800–804. [PubMed: 15761071]
5. Polgreen PM, Fultz SL, Justice AC, Wagner JH, Diekema DJ, Rabeneck L, Weissman S, et al. Association of hypocholesterolaemia with hepatitis C virus infection in HIV-infected people. *HIV Med.* 2004; 5:144–150. [PubMed: 15139979]
6. Jarmay K, Karacsony G, Nagy A, Schaff Z. Changes in lipid metabolism in chronic hepatitis C. *World J Gastroenterol.* 2005; 11:6422–6428. [PubMed: 16425410]
7. Visnegarwala F, Chen L, Raghavan S, Tedaldi E. Prevalence of diabetes mellitus and dyslipidemia among antiretroviral naïve patients co-infected with hepatitis C virus (HCV) and HIV-1 compared to patients without co-infection. *J Infect.* 2005; 50:331–337. [PubMed: 15845431]
8. Jan CF, Chen CJ, Chiu YH, Chen LS, Wu HM, Huang CC, Yen MF, et al. A population-based study investigating the association between metabolic syndrome and hepatitis B/C infection (Keelung Community-based Integrated Screening study No. 10). *Int J Obes (Lond).* 2006; 30:794–799. [PubMed: 16404404]
9. Siagris D, Christofidou M, Theocharis GJ, Pagoni N, Papadimitriou C, Lekkou A, Thomopoulos K, et al. Serum lipid pattern in chronic hepatitis C: histological and virological correlations. *J Viral Hepat.* 2006; 13:56–61. [PubMed: 16364083]
10. Bedimo R, Ghurani R, Nsuami M, Turner D, Kvanli MB, Brown G, Margolis D. Lipid abnormalities in HIV/hepatitis C virus-coinfected patients. *HIV Med.* 2006; 7:530–536. [PubMed: 17105512]
11. Petit JM, Benichou M, Duvillard L, Jooste V, Bour JB, Minello A, Verges B, et al. Hepatitis C virus-associated hypobetalipoproteinemia is correlated with plasma viral load, steatosis, and liver fibrosis. *Am J Gastroenterol.* 2003; 98:1150–1154. [PubMed: 12809841]
12. Serfaty L, Andreani T, Giral P, Carbonell N, Chazouilleres O, Poupon R. Hepatitis C virus induced hypobetalipoproteinemia: a possible mechanism for steatosis in chronic hepatitis C. *J Hepatol.* 2001; 34:428–434. [PubMed: 11322205]
13. Grigorescu M, Rusu M, Neculoiu D, Radu C, Serban A, Catanas M, Grigorescu MD. The FibroTest Value in Discriminating between Insignificant and Significant Fibrosis in Chronic Hepatitis C Patients. The Romanian Experience. *J Gastrointest Liver Dis.* 2007; 16:31–37. [PubMed: 17410286]
14. Myers RP, Benhamou Y, Imbert-Bismut F, Thibault V, Bochet M, Charlotte F, Ratzu V, et al. Serum biochemical markers accurately predict liver fibrosis in HIV and hepatitis C virus co-infected patients. *Aids.* 2003; 17:721–725. [PubMed: 12646795]
15. Imbert-Bismut F, Ratzu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet.* 2001; 357:1069–1075. [PubMed: 11297957]
16. Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, et al. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J Hepatol.* 2007; 46:403–410. [PubMed: 17126448]
17. Soardo G, Pirisi M, Fonda M, Fabris C, Falleti E, Toniutto P, Vitulli D, et al. Changes in blood lipid composition and response to interferon treatment in chronic hepatitis C. *J Interferon Cytokine Res.* 1995; 15:705–712. [PubMed: 8528943]
18. Toyoda H, Kumada T. Cholesterol and lipoprotein levels as predictors of response to interferon for hepatitis C. *Ann Intern Med.* 2000; 133:921. [PubMed: 11103068]
19. Minuk GY, Weinstein S, Kaita KD. Serum cholesterol and low-density lipoprotein cholesterol levels as predictors of response to interferon therapy for chronic hepatitis C. *Ann Intern Med.* 2000; 132:761–762. [PubMed: 10787379]
20. Gopal K, Johnson TC, Gopal S, Walfish A, Bang CT, Suwandhi P, Pena-Sahdala HN, et al. Correlation between beta-lipoprotein levels and outcome of hepatitis C treatment. *Hepatology.* 2006; 44:335–340. [PubMed: 16871569]

21. Economou M, Milionis H, Filis S, Baltayiannis G, Christou L, Elisaf M, Tsianos E. Baseline cholesterol is associated with the response to antiviral therapy in chronic hepatitis C. *J Gastroenterol Hepatol*. 2008; 23:586–591. [PubMed: 17498221]
22. Mawatari H, Yoneda M, Fujita K, Nozaki Y, Shinohara Y, Sasaki H, Iida H, et al. Association between lipoprotein subfraction profile and the response to hepatitis C treatment in Japanese patients with genotype 1b. *J Viral Hepat*. 2009
23. Sheridan DA, Price DA, Schmid ML, Toms GL, Donaldson P, Neely D, Bassendine MF. Apolipoprotein B-associated cholesterol is a determinant of treatment outcome in patients with chronic hepatitis C virus infection receiving anti-viral agents interferon-alpha and ribavirin. *Aliment Pharmacol Ther*. 2009; 29:1282–1290. [PubMed: 19392865]
24. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature*. 2009
25. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet*. 2009; 41:1105–1109. [PubMed: 19749757]
26. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet*. 2009; 41:1100–1104. [PubMed: 19749758]
27. Borden EC, Rosenzweig IB, Byrne GI. Interferons: from virus inhibitor to modulator of amino acid and lipid metabolism. *J Interferon Res*. 1987; 7:591–596. [PubMed: 2445857]
28. Schectman G, Kaul S, Mueller RA, Borden EC, Kissebah AH. The effect of interferon on the metabolism of LDLs. *Arterioscler Thromb*. 1992; 12:1053–1062. [PubMed: 1525120]
29. White IR, Patel K, Symonds WT, Dev A, Griffin P, Tsokanas N, Skehel M, et al. Serum proteomic analysis focused on fibrosis in patients with hepatitis C virus infection. *J Transl Med*. 2007; 5:33. [PubMed: 17625010]
30. Stuyver L, Rossau R, Wyseur A, Duhamel M, Vanderborght B, Van Heuverswyn H, Maertens G. Typing of hepatitis C virus isolates and characterization of new subtypes using a line probe assay. *J Gen Virol*. 1993; 74(Pt 6):1093–1102. [PubMed: 8389799]
31. Livak KJ. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. *Genet Anal*. 1999; 14:143–149. [PubMed: 10084106]
32. McCarthy JJ, Li JH, Thompson AJ, Suchindran S, Lao XQ, Goldstein D, Patel K, et al. Replication of an interferon lambda genetic polymorphism strongly associated with treatment response in patients with chronic hepatitis C infection in a tertiary care setting. *in review*.
33. Hegele RA. Plasma lipoproteins: genetic influences and clinical implications. *Nat Rev Genet*. 2009; 10:109–121. [PubMed: 19139765]
34. Chen L, Borozan I, Feld J, Sun J, Tannis LL, Coltescu C, Heathcote J, et al. Hepatic gene expression discriminates responders and nonresponders in treatment of chronic hepatitis C viral infection. *Gastroenterology*. 2005; 128:1437–1444. [PubMed: 15887125]
35. Sarasin-Filipowicz M, Oakeley EJ, Duong FH, Christen V, Terracciano L, Filipowicz W, Heim MH. Interferon signaling and treatment outcome in chronic hepatitis C. *Proc Natl Acad Sci U S A*. 2008; 105:7034–7039. [PubMed: 18467494]
36. Ehnholm C, Aho K, Huttunen JK, Kostiaainen E, Mattila K, Pakkarainen J, Cantell K. Effect of interferon on plasma lipoproteins and on the activity of postheparin plasma lipases. *Arteriosclerosis*. 1982; 2:68–73. [PubMed: 6174110]
37. Shinohara E, Yamashita S, Kihara S, Hirano K, Ishigami M, Arai T, Nozaki S, et al. Interferon alpha induces disorder of lipid metabolism by lowering postheparin lipases and cholesteryl ester transfer protein activities in patients with chronic hepatitis C. *Hepatology*. 1997; 25:1502–1506. [PubMed: 9185775]
38. Andrade RJ, Garcia-Escano MD, Valdivielso P, Alcantara R, Sanchez-Chaparro MA, Gonzalez-Santos P. Effects of interferon-beta on plasma lipid and lipoprotein composition and post-heparin lipase activities in patients with chronic hepatitis C. *Aliment Pharmacol Ther*. 2000; 14:929–935. [PubMed: 10886050]
39. Ank N, West H, Paludan SR. IFN-lambda: novel antiviral cytokines. *J Interferon Cytokine Res*. 2006; 26:373–379. [PubMed: 16734557]

40. Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. *Circulation*. 2008; 118:2047–2056. [PubMed: 18955664]
41. Harrison SA, Rossaro L, Hu KQ, Patel K, Tillmann HL, Dhaliwal S, Torres DM, et al. Relationship of the use of statins and elevated low density lipoprotein (LDL) or total cholesterol (TC) to virologic response in patients treated for hepatitis C virus (HCV) in the IDEAL study. *Hepatology*. 2009; 50(suppl):360A.
42. Ye J. Reliance of host cholesterol metabolic pathways for the life cycle of hepatitis C virus. *PLoS Pathog*. 2007; 3:e108. [PubMed: 17784784]
43. Agnello V, Abel G, Elfahal M, Knight GB, Zhang QX. Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. *Proc Natl Acad Sci U S A*. 1999; 96:12766–12771. [PubMed: 10535997]
44. Andre P, Komurian-Pradel F, Deforges S, Perret M, Berland JL, Sodoyer M, Pol S, et al. Characterization of low- and very-low-density hepatitis C virus RNA-containing particles. *J Virol*. 2002; 76:6919–6928. [PubMed: 12072493]
45. Molina S, Castet V, Fournier-Wirth C, Pichard-Garcia L, Avner R, Harats D, Roitelman J, et al. The low-density lipoprotein receptor plays a role in the infection of primary human hepatocytes by hepatitis C virus. *J Hepatol*. 2007; 46:411–419. [PubMed: 17156886]
46. Maillard P, Huby T, Andreo U, Moreau M, Chapman J, Budkowska A. The interaction of natural hepatitis C virus with human scavenger receptor SR-BI/Cla1 is mediated by ApoB-containing lipoproteins. *Faseb J*. 2006; 20:735–737. [PubMed: 16476701]
47. Ye J, Wang C, Sumpter R Jr, Brown MS, Goldstein JL, Gale M Jr. Disruption of hepatitis C virus RNA replication through inhibition of host protein geranylgeranylation. *Proc Natl Acad Sci U S A*. 2003; 100:15865–15870. [PubMed: 14668447]

List of Abbreviations

LDL	low density lipoprotein
HCV	hepatitis C virus
VLDL	very low density lipoprotein
HIV	human immunodeficiency virus
PEG-IFN	pegylated interferon
RBV	ribavirin
SVR	sustained virologic response
SNP	single nucleotide polymorphism
Apo	apolipoprotein

Figure 1. Differences in LDL cholesterol, apolipoprotein B levels, and total cholesterol by HCV genotype in patients with chronic HCV infection

Panel (A) LDL cholesterol; Panel (B) Apolipoprotein B; Panel (C) Total cholesterol. Means and standard error bars shown, adjusted for cohort, age, sex, and race.

Figure 2. Effect of rs12979860 genotype (CC versus TT/TC) on levels of LDL cholesterol and apolipoprotein B, stratified by HCV genotype (gt 1, gt 2, gt 3)
 Panel (A) LDL cholesterol (LDL-C); Panel (B) Apolipoprotein B. Means and standard error bars shown, adjusted for cohort, age, sex, and race. Pooled analysis was additionally controlled for HCV genotype.

Table 1
Characteristics of chronic HCV patients in Duke and Farglitazar cohorts

Variable	Duke (N=634)	Farglitazar (N=112)	
Sex, male (%)	391 (62)	72 (64)	
Race (%)			‡
Caucasian	452 (71)	100 (89)	
African-American	182 (29)	12 (11)	
METAVIR fibrosis stage (%), N=633			‡
F0-1	146 (28)	40 (36)	
F2	203 (39)	54 (49)	
F3-4	173 (33)	17 (15)	
HCV genotype (%)			‡
1	539 (85)	112 (100)	
2/3	95 (15)	0 (0)	
rs12979860 genotype (%)			
TT	127 (20)	23 (21)	
TC	322 (51)	60 (54)	
CC	185 (29)	29 (26)	
	<i>Mean ± SD</i> *	<i>Mean ± SD</i>	
Age in years	49.5 ± 8.5	51.5 ± 6.9	
Body Mass Index (kg/m ²), N=664	28.8 ± 5.7	28.9 ± 4.5	
Total cholesterol (mg/dL), N=577	175.0 ± 40.8	--	
HDL-C (mg/dL), N=577	47.4 ± 16.4	--	
LDL-C (mg/dL)	102.0 ± 35.7	94.0 ± 30.8	‡
Apo A-I (mg/dL)	137.0 ± 29.9	138.7 ± 29.2	
Apo B (mg/dL)	79.0 ± 23.4	72.4 ± 18.6	‡
	<i>Median (IQR)</i> †	<i>Median (IQR)</i>	
Triglycerides† (mg/dL)	115.6 (2.0)	95.6 (1.7)	‡
Apo C-III† (mg/dL)	9.2 (1.6)	10.8 (1.5)	‡
Apo E† (mg/dL), N=577	4.2 (1.5)	--	

* standard deviation;

† interquartile range;

‡ denotes significant (p<0.01) difference between populations

Table 2
Association between rs12979860 genotype and lipid levels in 746 chronic HCV patients

Lipid	TT/TC (N=532)	CC (N=214)	P
Total cholesterol* (mg/dL)	168.5 ± 3.7	181.7 ± 4.5	6.0×10⁻⁴
Triglycerides [†] (mg/dL)	105.6 ± 6.3	93.1 ± 7.3	0.03
HDL-C* (mg/dL)	47.6 ± 1.4	47.2 ± 1.7	0.78
LDL-C (mg/dL)	92.6 ± 3.3	110.3 ± 3.9	8.9×10⁻¹⁰
Apo C-III [†] (mg/dL)	10.4 ± 0.4	10.1 ± 0.5	0.74
Apo A-I (mg/dL)	141.0 ± 2.6	136.8 ± 3.1	0.06
Apo B (mg/dL)	71.1 ± 2.2	80.3 ± 2.5	1.3×10⁻⁶
Apo E ^{†*} (mg/dL)	4.4 ± 0.1	4.0 ± 0.2	0.01

Values are reported as least-squares mean ± standard error and are adjusted for cohort, age, sex, race, and HCV genotype.

[†] Normalized by log transformation before analysis.

* Data available only on a subset of the population (117 TT, 299 TC, 161 CC).

Table 3
Odds ratios associated with LDL cholesterol and rs12979860 as predictors of SVR to PEG-IFN/RBV among 182 chronic HCV genotype 1 patients with complete data

Predictor	Adj. OR ¹ (95% CI)	P	Adj OR ² (95% CI)	P
LDL-C (per mg/dL)	2.01 (1.32-3.05)	1.1×10⁻³	1.56 (0.99-2.47)	0.06
rs12979860 CC	8.11 (3.05-21.58)	2.8×10⁻⁵	5.81 (2.07-16.25)	8.0 ×10⁻⁴

Adjusted odds ratio 1 (Adj. OR¹) included either LDL cholesterol or rs12979860 genotype along with the following covariates: age, sex, race, and fibrosis. Adjusted odds ratio 2 (Adj. OR²) included both LDL cholesterol and rs12979860 genotype in the model with the other covariates. Odds ratios are for LDL cholesterol (per one standard deviation increase) and rs12979860 (CC vs CT/TT).