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ORIGINAL ARTICLE

Association of IL28B genotypes with metabolic profiles and viral clearance rate in chronic hepatitis C patients

Ching-Sheng Hsu · Shih-Jer Hsu · Hung-Chia Chen · Chen-Hua Liu · Jenher Jeng · Chun-Jen Liu · Pei-Jer Chen · Ding-Shinn Chen · Jia-Horng Kao

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Abstract
Purpose IL28B genotypes have a strong impact on treatment outcomes of chronic hepatitis C (CHC) and on-treatment viral kinetics. Since metabolic regulation and interferon response are highly integrated, metabolic profiles may play an important role in the link between IL28B genotypes and hepatitis C virus (HCV) infection. Thus, the association of IL28B rs8099917 genotypes with metabolic profiles and the impact of metabolic profiles on hepatitis C viral kinetic parameters were examined.
Methods A case–control analysis including 278 CHC patients and 280 subjects without chronic HCV infection was performed. The associations of IL28B rs8099917 genotype with pretreatment metabolic profiles and early viral kinetic parameters were evaluated.
Results Compared to HCV genotype 1 patients, the differences in metabolic profiles were more significant in genotype 2 patients. HCV genotype 2 patients with TT genotype had higher serum total cholesterol and high density lipoprotein (HDL) levels than those with GT genotype, and the differences remained significant when adjusted for age, sex, and body mass index ($p = 0.005$ for total cholesterol; $p = 0.006$ for HDL). In addition, patients with higher serum TG, higher fasting blood glucose, and lower HDL had a lower viral clearance rate.
Conclusions IL28B genotypes may affect lipid profiles of CHC patients, especially in HCV-genotype 2 patients. Patients with higher serum fasting blood glucose, triglyceride, and lower HDL have a lower viral clearance rate during pegylated interferon plus ribavirin therapy.

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C.-S. Hsu
Division of Gastroenterology, Department of Internal Medicine, Buddhist Tzu Chi General Hospital, Taipei, Taiwan

C.-S. Hsu
School of Medicine, Tzu Chi University, Hualien, Taiwan

S.-J. Hsu · C.-H. Liu · C.-J. Liu · P.-J. Chen · D.-S. Chen · J.-H. Kao (✉)
Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, National Taiwan University Hospital, Taipei, Taiwan
e-mail: kaojh@ntu.edu.tw

S.-J. Hsu
Department of Internal Medicine, National Taiwan University Hospital, Yun-Lin Branch, Yun-Lin, Taiwan

H.-C. Chen
Division of Personalized Nutrition and Medicine, National Center for Toxicological Research, FDA, Jefferson, USA

C.-H. Liu · C.-J. Liu · P.-J. Chen · D.-S. Chen · J.-H. Kao
Department of Internal Medicine, National Taiwan University College of Medicine, National Taiwan University Hospital, Taipei, Taiwan

J. Jeng
Center of Mathematical Modeling and Scientific Computing, National Chiao Tung University, Hsinchu, Taiwan

P.-J. Chen · J.-H. Kao
Hepatitis Research Center, National Taiwan University College of Medicine, National Taiwan University Hospital, Taipei, Taiwan

J.-H. Kao
Department of Medical Research, National Taiwan University College of Medicine, National Taiwan University Hospital, Taipei, Taiwan

Keywords HCV RNA · Hepatitis C virus · IL28B genotype · Mathematical model · Metabolic profiles · Pegylated interferon · Viral kinetics

Abbreviations

HCV	Hepatitis C virus
CHC	Chronic hepatitis C
Peg-IFN	Pegylated interferon
ALT	Alanine aminotransferase
GGT	Gamma-glutamyl transpeptidase
WBC	White blood count
PLT	Platelet count
BMI	Body mass index
ULN	Upper limit of normal
RVR	Rapid virologic response
EVR	Early virologic response
SVR	Sustained virologic response
SNP	Single nucleotide polymorphism
TG	Triglyceride
TC	Total cholesterol
HDL	High density lipoprotein
LDL	Low density lipoprotein
IR	Insulin resistance

Introduction

Hepatitis C virus (HCV) affects more than 180 million people worldwide and is the major etiology of chronic liver diseases, such as hepatitis, cirrhosis, and hepatocellular carcinoma [1, 2]. Therefore, tackling HCV effectively is an important health issue globally. However, interferon (IFN)-based treatment for chronic hepatitis C (CHC) is expensive and only effective in a certain proportion of patients and has many unpleasant adverse effects [3, 4]. Therefore, practicing physicians need to use several predictors to access the benefits and risks of CHC therapy and advise patients who are likely to respond to continue the treatment or stop treatment in those who may fail. Among these predictors, on-treatment viral kinetics [5] and metabolic factors [6, 7] have been recognized as important factors predictive of sustained virologic response (SVR) to IFN-based therapy [8, 9].

Of note, two single nucleotide polymorphisms (SNPs) of the IL28B gene encoding IFN-lambda-3 (IFN-lambda-3), rs8099917, and rs12979860, have been documented to have strong associations with spontaneous clearance of HCV and virologic responses to IFN-based therapy, which may explain the differences in SVR rates among African-Americans, Europeans, and Asians [10–14]. Our previous study using a new mathematical model also highlighted that Asian CHC patients with rs8099917 TT genotype may have an increasing viral clearance rate [15]. Therefore, simultaneous consideration of viral kinetics, metabolic factors, and SNPs of IL28B gene has become the core in our daily clinical practice for HCV management.

Although IL28B genotype may partly explain the relationship of hepatic IFN-stimulated genes (ISGs) expression and treatment outcome of CHC [16–18], the link between IL28B genotype and ISGs is not observed in subjects who were not infected with HCV. For examples, our recent study did not show the link between IL28B and treatment outcome of chronic hepatitis B [19]. Moreover, a study also identified an independent relationship between IL28B genotype and hepatic expression of ISGs in CHC patients receiving IFN-based treatment [16]. Therefore, the mechanisms by which IL28B genetic variations affect treatment outcome of CHC remain unclear and deserve further studies. Since metabolic regulation and immune response are highly integrated, metabolic factors may play a certain role in this association. To this end, we examined the association between IL28B rs8099917 genotype and metabolic profiles in HCV-infected and non-infected subjects. In addition, the impacts of metabolic profiles on early viral kinetic parameters following IFN-based therapy were studied using a mathematical model.

Patients and methods

HCV group

A total of 278 consecutive CHC patients were enrolled from the gastroenterological clinics of the National Taiwan University Hospital and its Yun-Lin branch. Among them, 91 CHC patients were selected from a previous prospective study of factors affecting early viral load decline during treatment with combination therapy for estimation of viral kinetic parameters [5]. In brief, chronic HCV infection was defined as the positivity of both anti-HCV and serum HCV RNA for more than 6 months. All patients had available histologic data, naive to IFN treatment and other experimental antiviral or immunosuppressive therapy. They had serum alanine aminotransferase (ALT) levels at least twice the upper limit of normal (ULN) on two occasions within the previous 6 months. None of them were positive for hepatitis B surface antigen or human immunodeficiency virus antibody or had a known history or serological evidence of autoimmune liver disease, inheritable disorders, such as hemochromatosis or Wilson's disease, renal insufficiency, malignancy, and a history of daily alcohol consumption >20 g or active drug abuse.

HCV genotype 1 patients received Peg-IFN alpha-2a 180 μg plus ribavirin for 48 weeks and genotype 2 patients

received Peg-IFN alpha-2a 180 μg plus ribavirin for 24 weeks, and all patients were followed for 24 weeks after discontinuation of treatment. One dose of subcutaneous Peg-IFN alpha-2a 180 μg was given at the beginning of the study (day 0). Then, Peg-IFN alpha-2a 180 μg plus ribavirin were administered from week 1 to week 24 or 48. The dosage of oral ribavirin was adjusted according to body weight (1,000 mg for weight ≤75 kg and 1,200 mg for weight >75 kg). Scheduled blood sampling was performed for HCV RNA detection and quantification. Serum HCV RNA levels were quantified before the first dosing (0 h), 4 h, 8 h, 12 h post first dosing on day 0, and daily for 3 consecutive days (days 1 to 3) in week 0, then at week 4, week 12, week 24, and every 24 weeks till the end of follow-up.

Uninfected control group

A total of 280 healthy controls negative for anti-HCV were enrolled simultaneously from the database of Health Management Center in the Buddhist Tzu Chi General Hospital, Taipei Branch, between 2006 and 2007. We excluded those positive for HBsAg, HIV, or those with insufficient information regarding HBsAg, anti-HCV, and HIV. Random selection without replacement was used to ensure that no control subject was assigned to more than once.

The primary outcome of interest was the association of metabolic profiles and IL28B rs8099917 genotype between different HCV genotypes and non-HCV controls.

Ethical considerations

The study was conducted in accordance with the principles of the Declaration of Helsinki, and approved by the Ethical Committee of the National Taiwan University Hospital and Buddhist Tzu Chi General Hospital, Taipei Branch. All patients gave informed consents before enrollment, and their viral parameters, biochemical, serologic as well as anthropometric data were recorded at enrollment.

Demographic and metabolic features

Information on gender, age, body mass index (BMI), serum fasting blood glucose, triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), aspartate aminotransferase (AST), and ALT levels was collected. BMI was calculated as weight in kilograms divided by height in square meters. Blood samples were collected in the morning after 12 h fasting and were measured by standard laboratory techniques in both cohorts. Serum fasting blood glucose, TG, TC, LDL, HDL, AST, and ALT levels were measured by an autoanalyzer (Hitachi 7250, Special; Hitachi, Tokyo, Japan). Serum insulin was measured by an immunometric assay (Immulite; Diagnostic Products Co., Los Angeles, CA, USA), according to the manufacturer's instructions.

Central obesity was defined as waist circumference ≥90 cm in men and ≥80 cm in women [20]. Fasting plasma glucose (FPG) level ≥100 mg/dL (5.6 mmol/L) but <126 mg/dl (7.0 mmol/L) was designated as impaired fasting glucose (IFG), according to the latest American Diabetes Association criteria [21]. ULN of serum ALT level was set at 30 IU/L for men and 19 IU/L for women [22, 23]. Upper limit of HDL was set at 40 mg/dL for men and 50 mg/dL for women [24].

Serological markers

Hepatitis B surface antigen (HBsAg) and anti-HCV were assayed with commercial kits (Abbott Laboratories, North Chicago, IL, USA).

Determination of insulin resistance

Insulin resistance (IR) index was determined using homeostasis model assessment (HOMA-IR = fasting insulin (mU/L) × fasting glucose (mg/dL) × 0.05551/22.5), as previously described [25, 26].

Extraction, quantification, and genotyping of HCV RNA

Serum RNA was extracted using a commercial kit (QIAamp RNA Blood Mini Kit; Qiagen Inc, Valencia, CA, USA). Serum HCV RNA level was quantified by the LightCycler (Roche Diagnostics Applied Science, Penzberg, Germany) with the detection limit of 86 copies/mL (i.e., 34 IU/mL) [27]. Genotyping of HCV was performed by the LightCycler PCR assay or reverse transcription-PCR (RT-PCR) with type-specific primers, as previously described [28–30]. The detection limit of type-specific primers genotyping method is 100 copies/mL (i.e., 37 IU/mL). All samples were tested in triplicate.

Extraction of genomic DNA and rs8099917 genotyping

All enrolled subjects were genotyped for the SNP (rs8099917). All their blood specimens were collected into ethylenediaminetetraacetic acid (EDTA) tubes. Genomic DNA was extracted by standard protocols with blood RBC lysis, cell lysis, DNA binding, wash and elution. Extracted DNA normalized to 50 ng/μL was obtained. DNA

quality was assessed by calculating the absorbance ratio OD260/280 nm using NanoDrop model ND-1000 (Thermo Scientific, Wilmington, DE, USA).

The SNP (rs8099917) was genotyped using the ABI TaqMan allelic discrimination kit and the ABI7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA).

Mathematical modeling of viral kinetics

The data were analyzed using previously published mathematical model for the effect of IFN-α on HCV dynamics [31, 32]. The model differential equations are as follows:

$$dT/dt = s - dT - (1-\eta)\beta VT + qI. \quad (1)$$

$$dI/dt = (1-\eta)\beta VT - kI - qI. \quad (2)$$

$$dV/dt = (1-\varepsilon)\, pI - cV. \quad (3)$$

In this model, T represents the number of target cells, I represents the number of productively infected cells, and V is the viral load. Target cells are produced at rate s and die with a death rate constant d. Cells become productively infected with de novo infection rate constant β and, once infected, may be killed with rate constant k or become nonproductive with rate constant q. HCV virions are produced by infected cells at an average rate of p virions per cell per day and are cleared with a clearance rate constant c. In this model, IFN-α is assumed to reduce virion production by infected cells by a fraction $(1-\varepsilon)$ but could also reduce de novo infection of target cells by the factor $(1-\eta)$. Before IFN-α therapy, $\varepsilon = \eta = 0$. At $t = t_0$, in which $t = 0$ is the time of the first injection and t_0 is a delay caused possibly because of a pharmacokinetic lag, IFN-α is assumed to block virus production $(0 \le \varepsilon \le 1)$ and to block de novo infection $(0 \le \eta \le 1)$. Assuming that the number of target cells does not change significantly over the course of the first weeks of treatment $[T(t) = T_0]$, it is not possible to uncouple the effects of killing infected cells (k) or making them nonproductive (q), and one needs to use a combined parameter $\delta = k + q$ depicting the total loss rate of productively infected cells. By differentiating the change rate of viral load (dV/dt) and assuming all the parameters are constant except for viral load and number of productively infected cells, we can obtain the acceleration of viral load according to Eqs. (2) and (3):

$$d^2V/dt^2 = -(c+\delta)dV/dt - [\delta c - (1-\varepsilon)p(1-\eta)\beta T]V \quad (4)$$

For a reasonable assumption that the number of target cells T will remain constant in the first 2 weeks after the first injection of IFN-α, there will be two possible analytical solutions for the second order differential equation:

(i) $V = K_1 e^{s1t} + K_2 e^{s2t}$;

or

(ii) $V = (K_1' t + K_2')e^{s't}$.

For (i):

$$S1 = -0.5(c+\delta) - 0.5[(c-\delta)^2 + 4(1-\varepsilon)p(1-\eta)\beta T]^{1/2},$$

$$S2 = -0.5(c+\delta) + 0.5[(c-\delta)^2 + 4(1-\varepsilon)p(1-\eta)\beta T]^{1/2}.$$

The S1 and S2 can be approximated by the $-c$ and $-\delta$ as ε and η are close to 1.

For $V = (K_1' + K_2')e^{s't}$ will be valid only under the condition that $(c-\delta)^2 + 4(1-\varepsilon)p(1-\eta)\beta T = 0$, thus if (ii) is valid, $c = \delta$ and $\varepsilon = 1$ or $\eta = 1$ should simultaneously exist, and finally $s' = -0.5(c+\delta) = -c = -\delta$. By differentiating V and comparing to Eq. (3), it shows that only the condition, $c = \delta$ and $\eta = 1$, is valid. Otherwise, the elevation of the virus loads can be interpreted under the condition, and thus we only fit this model.

Nonlinear data fitting

The estimation by fitting log of viral loads is not included for further analyses because it will eliminate the immediately slight increasing effect of the viral loads after first dosing. To estimate HCV viral kinetic parameters, the $V(t)$ from the analytical solution [Eq. (4)] is fit to the viral load data of the first 3 days, individually for each patient by a nonlinear least squares method using R(version 2.10.0, The R Foundation for Statistical Computing), fitting simultaneously K_1', K_1', and s' (assuming $t_0 = 0$).

Statistical analysis

Categorical data were presented as percentages, while continuous data were presented as mean with standard deviations. Log transformation was performed for variables with a significant deviation from normal distribution. Chi-square tests, t tests, and Wilcoxon's tests were used to analyze categorical, parametric continuous, and non-parametric variables, respectively. The multivariate regression analyses were obtained by the linear regression models. Multi-factor ANOVA is used to adjust the possible significant effects of the other variables such as age, sex and BMI. All analyses were performed with R(version 2.10.0, The R Foundation for Statistical Computing). All tests were two-sided and $p < 0.05$ was considered statistically significant, unless mentioned otherwise.

Results

Baseline characteristics of subjects

A total of 278 CHC patients and 280 non-HCV controls with available IL28B SNP (rs8099917) data were enrolled. The mean BMI value was 24.6 kg/m^2, which fell in the overweight range for the South–East Asia population as a whole, and the distributions of selected demographic characters were shown in Table 1. In brief, patients in the HCV group were older and predominantly male and had significantly higher fasting blood glucose, lower serum TG, TC, and LDL levels than the control group. Among the 278 CHC patients, 169 (61.2 %) had HCV genotype 1 infection, 95 (34.4 %) had genotype 2 infection, 12 (4.4 %) had mixed genotype 1 and genotype 2 infection, and 2 were unclassified.

Table 1 Comparisons of demographic and metabolic characteristics between 278 patients with chronic HCV infection and 280 non-HCV controls

	HCV patients, *n* (%)	Non-HCV controls, *n* (%)	*p* value
Age (years)	54.6 ± 10.9	52.5 ± 10.2	0.023
Male (%)	164 (59.0)	126 (45.0)	0.001
BMI (kg/m^2)	25.7 ± 3.8	23.5 ± 3.2	<0.001
Fasting blood glucose (mg/dL)	105.7 ± 31.3	98.7 ± 23.4	0.003
Triglyceride (mg/dL)	110.4 ± 66.8	114.5 ± 69.9	0.485
Total cholesterol (mg/dL)	170.9 ± 33.3	196.6 ± 33.9	<0.001
LDL (mg/dL)	102.9 ± 33.2	127.7 ± 30.5	<0.001
HDL (mg/dL)	46.2 ± 11.7	55.8 ± 16.0	<0.001
Albumin (g/dL)	4.2 ± 0.3	4.6 ± 0.3	<0.001
AST (U/L)	94.4 ± 69.9	23.9 ± 9.8	<0.001
ALT (U/L)	139.2 ± 115.2	24.9 ± 15.3	<0.001
WBC (K/UL)	5.3 ± 1.6	5.5 ± 1.6	0.055
Hb (g/dL)	14.5 ± 1.6	14.6 ± 1.4	0.305
PLT (K/UL)	171.9 ± 53.5	252.5 ± 81.6	<0.001
rs8099917 TT genotype (%)	247 (88.9)	252 (90.0)	0.685
HCV RNA Log_{10} (IU/mL)	5.8 ± 1.0	–	–
HCV genotype 1 (%)	169 (61.2)	–	
HOMA-IR[a]	4.0 ± 5.6		
METAVIR activity[b] (A0–1/A2–3)	172/38	–	
METAVIR fibrosis (F0–2/F3–4)	117/99		

Data is shown by mean ± standard error or case number (proportion)

BMI body mass index, *LDL* low-density lipoprotein-cholesterol, *HDL* high-density lipoprotein-cholesterol, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *WBC* white blood count, *Hb* hemoglobin, *PLT* platelet count, *HOMA-IR* homeostatic model assessment-insulin resistance

[a] There were 62 HCV genotype 1-infected patients, and 51 genotype 2-infected patients had available HOMA-IR index values

[b] Histologic data were evaluated semi-quantitatively using Metavir scoring system

Association of IL28B genotypes with metabolic profiles

The frequencies of IL28B genotypes were in Hardy–Weinberg equilibrium in the study population ($p = 0.8559$). Subjects with rs8099917 TT genotype had lower BMI (mean ± SD = 24.4 ± 3.5 kg/m^2) than those with GT genotype (25.8 ± 4.5 kg/m^2). The difference remained significant after adjustment for age and sex ($p = 0.003$). Subjects with TT genotype had higher HOMA-IR index values (mean ± SD = 4.3 ± 6.0) than those with GT genotype (2.7 ± 1.8), while the difference was not significant after adjustment for age, sex, and BMI ($p = 0.221$). However, the differences were not statistically significant in terms of serum TG, TC, LDL, HDL, and fasting blood glucose levels (Supplementary Table 1).

Differential effects of IL28B genotypes on metabolic profiles between HCV genotype 1 and genotype 2 subjects

As the associations of metabolic profiles between HCV genotype 1 and genotype 2 infection are different [6], we examined the interactions of HCV infection with IL28B genotypes and metabolic profiles according to HCV genotypes and divided HCV cohort into HCV genotype 1 and genotype 2 infected subjects, respectively. In comparison to HCV genotype 1 patients, the differences in metabolic profiles were more significant in genotype 2 patients (Table 2). HCV genotype 2 patients with TT genotype had higher TC and HDL levels than those with GT genotype, and the differences remained significant when adjusted for age, sex, and BMI ($p = 0.005$ for TC; $p = 0.006$ for HDL) (Table 2).

To explore the interaction between IL28B genotype and metabolic profiles, we constructed multivariate analytic models including the interaction term of IL28B SNP × HCV to examine the relationships among HCV infection, IL28B genotypes, and metabolic profiles. The results showed a significant interaction between HCV genotypes and IL28B genotypes on serum TC and HDL level ($p < 0.05$) (Table 3) and an obvious interaction between HCV and IL28B genotypes on metabolic profiles in patients with HCV genotype 2 infection (Supplementary Table 2).

Metabolic factors affecting viral kinetic parameters after IFN-based therapy

To estimate HCV viral kinetic parameters following IFN-based therapy, the *V* was fit to the serial viral loads of the

Table 2 Differential impacts of IL28B genotypes on metabolic profiles between HCV genotype 1 and 2 patients

	HCV genotype 1 ($n = 169$)			HCV genotype 2 ($n = 95$)		
	TT allele ($n = 150$)	GT allele ($n = 19$)	p value[a]	TT allele ($n = 84$)	GT allele ($n = 11$)	p value[a]
BMI (kg/m^2)	25.4 ± 3.8	27.3 ± 4.0	0.067	25.4 ± 3.4	27.0 ± 4.5	0.302
Fasting blood glucose (mg/dL)	107 ± 33.6	112.2 ± 38.2	0.588	101.6 ± 27.6	114.6 ± 29.6	0.191
Triglyceride (mg/dL)	114.9 ± 77.5	125.1 ± 43.5	0.396	102.7 ± 57.2	108.9 ± 36.4	0.629
Total cholesterol (mg/dL)	169.3 ± 35.3	173.2 ± 24.5	0.536	176.6 ± 33.1	150.5 ± 23.5	0.005
LDL (mg/dL)	102 ± 34.5	105.4 ± 22.4	0.579	105.2 ± 33.2	90.2 ± 29.2	0.140
HDL (mg/dL)	45.4 ± 11.6	45.8 ± 8.8	0.846	48.8 ± 12.3	41.4 ± 8.2	0.017
HOMA-IR[b]	5.3 ± 7	2.8 ± 2.1	0.045	3.1 ± 4.3	2.4 ± 1.4	0.442

According to this table shown above, serum total cholesterol and HDL levels are significantly different between IL28B genotypes in HCV genotype 2 patients

LDL low-density lipoprotein-cholesterol, *HDL* high-density lipoprotein-cholesterol, *HOMA* homeostasis model assessment, *IR* insulin resistance

[a] Student t test

[b] There were 62 HCV genotype 1-infected patients, and 51 genotype 2-infected patients had available HOMA-IR index values

Table 3 Linear regression models in 169 patients with HCV genotype 1 infection and 95 patients with HCV genotype 2 infection

	Log_{10} (triglyceride) (mg/dL)		Total cholesterol (mg/dL)		HDL (mg/dL)		LDL (mg/dL)	
	Coefficient	p	Coefficient	p	Coefficient	p	Coefficient	p
Intercept	1.51	<0.0001	160.5167	<0.0001	70.3274	<0.0001	86.9943	<0.0001
Sex (male vs. female)	0.0606	0.0183	−3.4356	0.4217	−5.6162	0.0001	1.145	0.7919
Age (years)	0.0009	0.4548	−0.0981	0.607	−0.1442	0.022	−0.0244	0.8992
BMI (kg/m^2)	0.0158	<0.0001	0.6355	0.2546	−0.5422	0.0034	0.6182	0.2758
SNP (rs8099917)	0.0546	0.2705	2.4654	0.7661	1.1879	0.6701	2.1793	0.7998
HCV genotype: genotype 2 = 1, genotype 1 = 0	−0.0333	0.2254	7.462	0.1052	3.7568	0.0134	3.0951	0.5067
SNP × HCV genotype	−0.0167	0.8365	−30.3962	0.026	−9.1556	0.0425	−17.9838	0.1951

The significant effect of SNP presenting in HCV genotype may be interpreted by the fitted model in this table, when main effect of SNP is equal to zero and the interaction effect of HCV genotype and SNP is unequal to 0. The former one is held in each model, but the later one is not. The associations between SNP and total cholesterol and HDL were significantly different between HCV genotypes 1 and 2

SNP single nucleotide polymorphism, *LDL* low-density lipoprotein-cholesterol, *HDL* high-density lipoprotein-cholesterol

first 3 days in 91 Taiwanese CHC patients receiving Peg-IFN alpha-2a plus ribavirin by a nonlinear least squares method, as previously described [15]. Patients with lower fasting blood glucose levels (<100 mg/dL) had higher viral clearance rate (mean ± SD = 4.12 ± 5.00/day) than those with higher fasting blood glucose level (≥100 mg/dL) (2.62 ± 1.99 mg/dL), and patients with higher serum HDL level (≥ULN) had higher viral clearance rate (mean ± SD = 4.23 ± 5.20/day) than those with lower serum HDL level (<ULN) (2.60 ± 1.77 mg/dL) (Table 4). Of note, patients with lower serum TG level (<100 mg/dL) had a higher viral clearance rate (mean ± SD = 4.42 ± 5.36/day) than those with higher serum TG level (≥100 mg/dL) (2.48 ± 1.34 mg/dL), and the difference remained significant when adjusted for age, sex, and BMI ($p < 0.05$) (Supplementary Table 3).

Discussion

In present study, we hypothesized that CHC patients with different IL28B genotypes may have different metabolic profiles, and through this linkage, CHC patients with different IL28B genotypes may have different therapeutic responses to IFN-based therapy. To that end, we constructed case–control analyses of subjects with and without chronic HCV infection. Our analyses not only demonstrated different metabolic associations between IL28B TT and GT genotype but also indicated a modifying effect of HCV infection on the link between IL28B genotypes and metabolic factors, especially in HCV genotype 2 patients. In addition, we also examined the effects of metabolic factors on viral kinetic parameters following IFN-based therapy in CHC patients and found those with higher serum

Table 4 The association of metabolic profiles with early viral kinetic parameters in chronic hepatitis C patients receiving pegylated interferon plus ribavirin therapy

			p value		
			t test	M–W test	K–S test
Fasting blood glucose (mg/dL)	<100	≥100			
Clearance rate	4.12 ± 5.00	2.62 ± 1.99	0.0483	0.0317	0.0467
Log_{10} production rate	10.71 ± 1.26	10.61 ± 0.96	0.6792	0.4060	0.3426
Triglyceride (mg/dL)	<100	≥100			
Clearance rate	4.42 ± 5.36	2.48 ± 1.34	0.0161	0.0461	0.0424
Log_{10} production rate	10.68 ± 1.10	10.67 ± 1.24	0.9754	0.9901	0.9678
Total cholesterol (mg/dL)	<200	≥200			
Clearance rate	3.29 ± 3.64	5.07 ± 6.45	0.3164	0.0313	0.0091
Log_{10} production rate	10.65 ± 1.14	10.78 ± 1.30	0.7212	0.6651	0.7214
LDL (mg/dL)	<130	≥130			
Clearance rate	3.43 ± 3.66	4.71 ± 6.65	0.4778	0.4876	0.8421
Log_{10} production rate	10.63 ± 1.24	10.86 ± 0.79	0.3722	0.6408	0.5996
HDL (mg/dL)	<UNL	≥UNL			
Clearance rate	2.60 ± 1.77	4.23 ± 5.20	0.0372	0.0750	0.1832
Log_{10} production rate	10.60 ± 1.15	10.70 ± 1.18	0.7041	0.5089	0.3595
HOMA-IR	<2.0	≥2.0			
Clearance rate	4.08 ± 4.47	3.14 ± 4.08	0.3062	0.0665	0.0692
Log_{10} production rate	10.64 ± 1.39	10.68 ± 0.91	0.8897	0.6868	0.3272

Male HDL ≥40 mg/dL and female HDL ≥50 mg/dL as HDL upper limits (ULN)

LDL low-density lipoprotein-cholesterol, *HDL* high-density lipoprotein-cholesterol, *HOMA* homeostasis model assessment, *IR* insulin resistance, *t test* Student's *t* test, *M–W test* Mann–Whitney *U* test, *K–S test* Kolmogorov–Smirnov test

TG and fasting blood glucose but lower HDL had a lower viral clearance rate. Taking these lines of evidence together, present study implied that the interaction with host metabolic factors may play an essential role on the link of IL28B genotypes and treatment outcome of CHC patients.

It is known that IL28B genotypes have a strong impact on treatment outcome of CHC [10–14] and on-treatment viral kinetics [15]. In addition, metabolic factors may also predict viral load declines as well as therapeutic response of CHC patients receiving IFN-based therapy [5, 33, 34]. Thus, if IL28B genotype affects treatment outcome of CHC through metabolic regulation, CHC patients with different IL28B genotypes may experience different metabolic profiles. To explore this important and interesting issue, we thus examined the association of IL28B genotype with metabolic factors between the HCV group and the control group. Since the associations of metabolic profiles between HCV genotype 1 and genotype 2 infection were different [6], we further examined the interactions of HCV infection with IL28B genotypes and metabolic profiles among different HCV genotypes. We found that there was no significant association between IL28B genotype and metabolic profiles in the control group, whereas significant associations were found between HCV and IL28B genotypes on serum metabolic profiles, especially in patients with HCV genotype 2 infection. As rs8099917 genotype may be associated with the expression of *IL28A* and/or *IL28B* in either whole blood [12] or peripheral blood mononuclear cells [13], and metabolic regulation are probably integrated with immune response [35], present study results were therefore not surprising. Accordingly, our data indeed implied a viral and HCV genotype-specific effect on this association, lent a strong support to the hypothesis that IL28B genotypes may affect host metabolic factors, and implied that HCV infection may modify the interaction between metabolic factors and IL28B genotypes. However, future studies are needed to confirm our findings.

Ample clinical and experimental evidences have linked metabolic factors, such as IR and serum lipid profiles, to HCV infection and its clinical outcomes [5, 33, 36, 37]. Although in vitro experiments showed lipid factors, including lipid rafts [38] and lipid droplet, are critical for producing infectious viruses [39] and that modifying the lipid factors may regulate HCV replication [40], there were still no obvious clinical evidences to support the modifying effects of metabolic factors on HCV replication. Nevertheless, several previous studies provided an indirect link between HCV replication and metabolic factors by demonstrating the proportional relationship between serum HCV RNA level and metabolic factors, such as IR [27, 41]

and lipid profiles [6]. In present study, we explored this issue using a viral kinetic approach, and compared viral kinetic parameters among different levels of metabolic profiles. Finally, we found that higher serum TG and fasting blood glucose, but lower HDL were associated with a lower viral clearance rate, but not viral production rate, implying that possible modifying effects of metabolic factors are mainly on serum hepatitis C viral clearance rather than HCV replication per se.

Present study had a few limitations. First, present study was a case–control design, and hence only the associations between chronic HCV infection and metabolic factors could be determined. However, this is the first study addressing the differential associations of IL28B genotypes between patients with chronic HCV infection and healthy adults. Second, although some studies have addressed the links of IL28B genotypes with lipid profiles in CHC patients [42, 43], whether such associations arise from HCV infection, IL28B genotype, or their interactions remain unclear. In present study, we not only demonstrated an association between IL28B genotypes with metabolic profiles but also indicated a modifying effect of HCV infection on this linkage in HCV genotype 2 patients. However, in contrast to prior studies, we did not identify a strong association between IL28B genotype and serum LDL levels among all HCV patients, while a significant association exists in HCV genotype 2 infected patients [42, 43]. The discrepancy may be due to different study population and design, as the previous studies included the ones who were mostly Caucasian, under non-fasting conditions, and some were non-responsive to prior IFN-based therapy or under treatment. Third, there was still no strong link observed between IL28B and non-HCV subjects [19], and a recent study indicated that IL28B genotype was independent to hepatic expression of ISGs in CHC patients receiving IFN-based treatment [16]. Therefore, though several studies already demonstrated the association of IL28B genotypes with ISGs in CHC patients [16–18], the link between IL28B genotypes and ISGs may be an epiphenomenon or at most attributable to the interaction between IL28B genotypes and HCV. The impact of IL28B genotypes on host metabolism may be a possible mechanism to explain this association. Lastly, the hypothesis is more valid for HCV genotype 2 infection; thus, mechanisms involving differential effect on HCV genotypes await further studies.

In conclusion, interactions among IL28B genotypes, metabolic factors, and HCV infection indeed exist. IL28B rs8099917 genotypes may affect lipid profiles of CHC patients, especially in HCV genotype 2 patients, but not in controls. In addition, elderly patients with higher serum fasting blood glucose and TG, but lower HDL, have a lower viral clearance rate following Peg-IFN plus ribavirin therapy. Therefore, the incremental viral clearance rate and better virologic response rate of patients with rs8099917 TT genotype may be partly reasoned by metabolic factors. Further studies are needed to explore the mechanisms involved in the interactions of IL28B genotypes and host metabolic factors with treatment-induced HCV clearance.

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