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Insulin resistance is independently associated with significant hepatic fibrosis in Asian chronic hepatitis C genotype 2 or 3 patients

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Abstract

Background and Aim: The role of insulin resistance (IR) and hepatic steatosis in fibrogenesis in chronic hepatitis C infection (CHC) has yielded conflicting data and few studies have been performed in Asian-region populations. We retrospectively investigated the relationship between host metabolic variables, including IR and hepatic steatosis, to hepatic fibrosis in Asian-region CHC genotype 2/3 patients.

Methods: A total of 303 treatment-naïve Asian-region patients with CHC genotype 2/3 were enrolled in a multicenter phase 3 study of albinterferon alfa-2b plus ribavirin for 24 weeks. IR was defined as Homeostasis Model for Assessment of IR (HOMA-IR) > 2. Baseline liver biopsy was evaluated by a single expert histopathologist. Post hoc subgroup logistic regression modeling selected for independent variables associated with significant fibrosis (METAVIR stage F2-F4).

Results: Insulin resistance was available in 263 non-diabetic Asian-region patients (hepatitis C virus-2 [HCV-2] = 171, HCV-3 = 92), and 433 non-Asian region patients (407 "Caucasian"); METAVIR fibrosis prevalence F0-F1 (minimal fibrosis) = 201 (77%) and F2-F4 (significant fibrosis) = 59 (23%), and steatosis prevalence of grade 0 = 169 (65%), grade 1 = 64 (25%), grade 2/3 = 27 (10%). Median HOMA-IR was 1.8 (interquartile range: 1.2–2.7); 100 (38%) patients had HOMA-IR > 2. Factors independently associated with significant fibrosis included HOMA-IR (odds ratio [OR] = 8.42), necro-inflammatory grade (OR = 3.17), age (OR = 1.07) and serum total cholesterol levels (OR = 0.008). This was similar to non-Asian region patients, but steatosis was not associated with significant fibrosis in either cohort.

Conclusions: In this subgroup study of Asian-region HCV genotype 2 or 3 patients, insulin resistance, along with age, cholesterol levels and necro-inflammation, but not steatosis may be associated with significant hepatic fibrosis.

Introduction

Chronic hepatitis C (CHC) infection induces insulin resistance (IR), hepatic steatosis and fibrogenesis through complex host-virus pathways that remain poorly defined.[1] Interference of insulin signaling through hepatitis C virus (HCV) core protein induced degradation of insulin receptor substrate-1 (IRS-1) represents one such pathway of IR development, and appears to be genotype-specific.[2] However, metabolic factors, cirrhosis, hepatic necro-inflammation, and hepatic steatosis may also contribute to the development of IR in CHC infection.[3] Several studies in predominantly Caucasian cohorts have now described an association between CHC and increased prevalence of IR and type 2 diabetes mellitus.[4] Insulin resistance is associated with HCV RNA levels for HCV genotypes-1 and -4,[5,6] and has also been associated with a reduced rate of sustained virologic response (SVR) in response

to antiviral therapy.[7–10] Importantly, IR is independently associated with fibrosis severity in CHC infection, likely through direct activation of pro-fibrogenic signaling pathways,[11,12] thus increasing the risk of disease progression,[13–17] and development of complications from end-stage liver disease.[18–20]

Interestingly, genotype-specific associations have also been observed with steatosis and fibrosis in CHC infection. HCV genotype 3 infection is associated with a viral-mediated steatosis that is often severe but appears to have different consequences to the steatosis associated with predominantly host metabolic risk factors seen in HCV genotype 1 infection.[21,22] Some studies have suggested that steatosis is associated with fibrosis for CHC genotype 3 infection,[23–26] and others report an association for CHC non-3 genotype infection only.[27,28] However, including insulin resistance in multivariate modeling appears to remove the independent effects of steatosis as a risk factor for fibrosis.[29]

Despite a relatively higher proportion of HCV genotype non-1 infection in Asian countries, and an increasing prevalence of metabolic syndrome and type II diabetes mellitus,[30,31] very few studies have evaluated the role of metabolic factors such as insulin resistance and steatosis on fibrosis in Asian CHC patients. These genotype-nonspecific cohort studies from Taiwan noted variable effects of IR and steatosis on advanced stage disease.[32,33] The influence of host metabolic factors on moderate-severe stage fibrosis from other Asian region CHC cohorts, or differences in these risk factors compared to Caucasian patients, has not been previously evaluated. Thus, our aims were to: (i) further examine associations between host metabolic factors, including IR and steatosis, to hepatic fibrosis in a large cohort of HCV genotype-2 or -3 patients from several countries in the Asian region; and (ii) assess for potential ethnic differences in metabolic risk factors for fibrosis by comparing these associations to non-Asian region patients.

Methods

Study population

Adult patients with HCV genotype 2/3 ($n = 932$, Asian region = 303) who had not previously received interferon-α (IFN-α) therapy were enrolled in a global phase III study of albinterferon alfa-2b conducted at 137 centers worldwide, including Asia (42), Australia (16), Europe (35), North America (41), and South America (3) between February 2007 and October 2008 (ClinicalTrials.gov number, NCT00411385).[34] Asian region patients were represented by six countries (India, Malaysia, Singapore, South Korea, Taiwan, and Thailand). Fasting samples were collected prospectively for measurement of baseline insulin resistance. All patients had compensated liver disease and no patient had evidence of other chronic liver disease, hepatocellular carcinoma, or alcohol dependence. Serum HCV-RNA levels were measured by real-time polymerase chain reaction (PCR) assay (COBAS Ampliprep/COBAS Taqman HCV Test, F. Hoffman-La Roche; limit of detection 15 IU/mL; lower limit of quantitation 43 IU/mL). HCV genotype was defined using Bayer TRUGENE HCV 5′NC Genotyping assay. All patients provided written informed consent according to the Declaration of Helsinki, with approval from the institutional review board at study sites.

Clinical and laboratory assessment

Clinical and biochemical evaluation for this study was performed at baseline, and included age, gender, race, and body mass index (BMI). Obesity was defined by BMI ≥ 30 in Caucasians and BMI ≥ 25 in Asians.[30] Fasting serum alanine aminotransferase (ALT), Gamma-glutamyltransferase (GGT), total cholesterol, triglycerides, and insulin resistance were measured at a central laboratory in all patients at baseline.

Insulin resistance

Insulin resistance was measured in fasting serum samples using the Homeostasis Model for Assessment of IR (HOMA-IR), calculated as fasting insulin (uU/mL) × fasting glucose (mmol/L)/22.5.[35] Insulin was measured by an electrochemiluminescence immunoassay (LabCorp, Burlington, NC, USA). There appear to be variable thresholds for HOMA-IR as a surrogate measure of insulin resistance, with levels of 1.8–2.3 reported in non-diabetic populations from Middle East and Southeast Asia.[36,37] A threshold of HOMA-IR > 2 was considered as the clinical definition of IR in our study. Given the variability in determination of an optimal threshold for HOMA-IR,[38] our multivariate modeling also considered HOMA-IR at a higher binary threshold of $> 3^6$ and as a continuous variable.

Liver histology

The baseline liver biopsy was centrally evaluated for METAVIR fibrosis stage, METAVIR inflammatory activity and hepatic steatosis grade by a single expert histopathologist (M.T.) who was blinded to all clinical and laboratory data. The METAVIR scoring system classifies fibrosis on a 5-point scale: F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = few septa; F3 = numerous septa without cirrhosis; and F4 = cirrhosis; necro-inflammatory activity is graded on a 4-point scale: A0 = none; A1 = mild; A2 = moderate; and A3 = severe.[39] Steatosis was graded as the percentage of hepatocytes containing macrovesicular fat droplets: grade 0 (0–5%), grade 1 (6–30%), grade 2 (31–60%) and grade 3 (≥ 61%).

Statistical analysis

For descriptive statistics, continuous variables were summarized as median (25–75th percentile) and categorical variables were described as frequency and percentage. Patients with missing data ($n = 162$) and diabetic patients were excluded ($n = 52$) from this post hoc subgroup study analysis. Despite log-transformation to approximate normality for skewed data for HOMA-IR, there remained a small number of significant outliers (> 2 standard deviations from the mean). Due to potential confounders relating to undiagnosed diabetes or non-fasting state, these outliers were also excluded from the analysis ($n = 22$). Comparisons between groups for baseline variables were performed using a Wilcoxon test for continuous variables. χ^2 test was used for categorical data. Significant fibrosis was classified as METAVIR stage F2-4. Multivariable linear and logistic regression modeling with backward selection was used to identify variables independently associated with significant fibrosis after adjustment for relevant covariates.

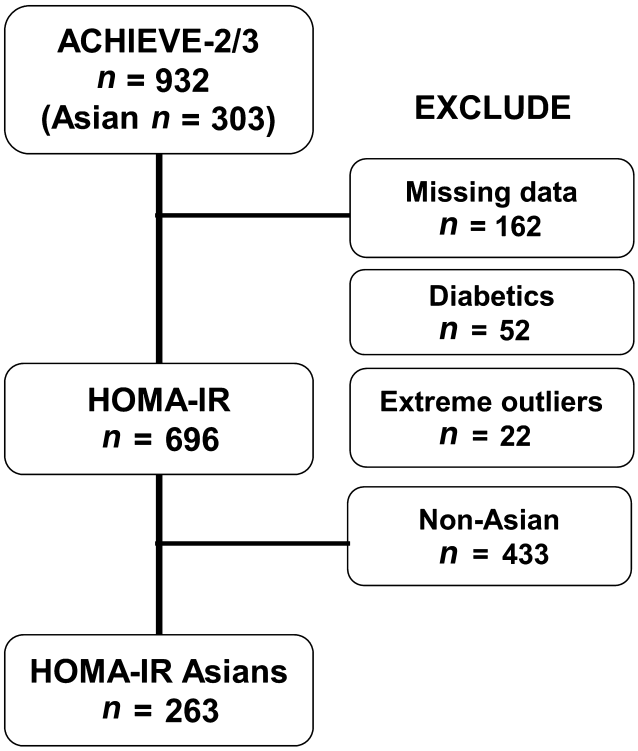

Figure 1 Flow chart of patients selected for this study. Following exclusion of patients with diabetes mellitus or incomplete data, 696 hepatitis C virus (HCV) genotype 2 or 3 patients ($n = 263$ Asian-region) had baseline Homeostasis Model for Assessment of insulin resistance (HOMA-IR) measurements available for analysis.

Biochemical variables were dichotomized for model building according to the upper limit of normal (ULN) for ALT, GGT, and fasting total cholesterol level. All statistical analyses were performed using SAS v9.1 (SAS institute, Cary, NC, USA), and significance assessed at $P < 0.05$ for all analyses.

Results

Patient characteristics

Baseline HOMA-IR and histology data were available in 696 patients (Asian region $n = 263$, Non-Asian $n = 433$) (Fig. 1). In the Asian region cohort ($n = 263$), there was a higher prevalence of genotype 2 ($n = 171$; 65%) compared to genotype 3 ($n = 92$, 35%) infection. Asian region patients with genotype 2 infection were older (age > 40 *vs* ≤ 40 years = 79% *vs* 49%, $P < 0.0001$), with higher total cholesterol (4.41 *vs* 3.77 mmol/L, $P < 0.0001$), marginally lower prevalence of elevated ALT (67% *vs* 78%, $P = 0.049$), and less steatosis (29% *vs* 49%, $P = 0.001$) than genotype 3 patients, but otherwise there were no significant differences between these two genotypes (Table 1). Most Asian region patients had minimal to mild stage fibrosis (METAVIR F0-F1, $n = 201$, 77%) (Fig. 2). Mean biopsy length was 13.4 ± 8.1 mm.

In comparison to Asian region patients, non-Asians were more likely to be obese and had a higher baseline median HOMA-IR (2.1 *vs* 1.8, $P = 0.0002$) (Table S1). Asian region patients had a greater prevalence of HCV genotype 2 infection compared to non-Asians (65% *vs* 36%, $P < 0.0001$), and were more likely to have significant liver fibrosis (23% *vs* 15%, $P = 0.0135$) (Table S1).

Insulin resistance in Asians

At a threshold of HOMA-IR > 2, IR was present in 100 of 263 (38%) patients from the Asian region. There were no significant differences in prevalence of IR between genotype 2 or 3 patients (Table 1). In this study, Asia region patients with IR were more likely to be obese (BMI > 30), to have hepatic steatosis and significant hepatic fibrosis (F2-4) (Table S2). There was no correlation between logHOMA-IR and baseline serum log HCV-RNA levels for with HCV genotype 2 (Rho = 0.008, $P = 0.2437$) or 3 (Rho = 0.032, $P = 0.0860$) infection.

Significant fibrosis

In multivariable analysis, factors independently associated with significant fibrosis in Asian region patients included $\log_{10}$HOMA-IR (odds ratio [OR] = 8.42, confidence interval [CI]: 2.1–34.3), lower fasting cholesterol (OR = 0.008, CI: < 0.001–0.205), necro-inflammatory activity (OR = 3.17, CI: 2.0–4.9) and age (OR = 1.065, CI: 1.03–1.1) (Table 2). In univariate analysis, there was a marginal association for BMI (OR 1.081, CI: 1.00–1.17, $P = 0.05$) but gender ($P = 0.85$), steatosis ($P = 0.12$) and genotype 2 or 3 ($P = 0.62$) were not associated with significant fibrosis in this cohort. In a multivariate logistic regression model considering binary variables, these same host factors (age > 40 years [OR = 6.5, CI: 2.5–16.7], moderate-to-severe necro-inflammation [OR = 4.23, CI: 2.1–8.6], total cholesterol < 130 mmol/L [OR = 2.95, CI: 1.3–6.7] and HOMA-IR ≥ 2 [2.76, CI: 1.4–5.3]) remained independently associated with stage F2-F4 fibrosis (Table 3). However, a higher threshold of HOMA-IR ≥ 3 ($n = 52/263$, 19.8%) was not independently associated with stage F2-F4 fibrosis, likely due to the smaller sample size.

For non-Asian region HCV genotype 2 or 3 patients ($n = 407$), age (OR = 1.04, CI: 1.04–1.1), $\log_{10}$HOMA-IR (OR = 3.36, CI: 1.1–10.2), lower fasting cholesterol (OR = 0.03, CI: 0.002–0.45) and necro-inflammatory activity (OR = 2.37, CI: 1.7–3.4) were also independently associated with stage F2-F4 fibrosis (Table 4).

Discussion

There is a relative paucity of published data in relation to pathogenesis of CHC infection in Asian patients. The prevalence of metabolic syndrome and type II diabetes mellitus has increased significantly in Asian countries in recent years, often present at a lower BMI threshold than observed in western countries.[30] With the caveat of data interpretation based on cross-sectional and post hoc subgroup analysis, this is the first large study to demonstrate an independent association between insulin resistance and significant fibrosis in an Asian-region cohort (representing six countries) with HCV genotype 2 or 3 infection. Host variables associated with significant fibrosis were similar between Asian region and non-Asian patients. The uniformity of data and sample collection, central laboratory assessments, and single pathologist evaluation represent significant strengths of this multi-center study. Inference

Table 1 Patient characteristics for Asian region patients by hepatitis C virus (HCV) genotype

	Genotype 2 ($n = 171$)	Genotype 3 ($n = 92$)	*P*-value
Male (*n*, %)	87 (51%)	54 (59%)	0.2253
Age > 40 (*n*, %)	135 (79%)	45 (49%)	< 0.0001
BMI (median, IQR)	24 (21–26)	24 (22–27)	0.2700
BMI > 30 kg/m^2 (*n*, %)	9 (5%)	9 (10%)	0.1663
BMI > 25 kg/m^2 (*n*, %)	64 (37%)	40 (43%)	0.3385
HOMA-IR (median, IQR)	1.7 (1.3–2.6)	1.8 (1.2–3.1)	0.6710
HOMA-IR > 2 (*n*, %)	63 (37%)	37 (40%)	0.5907
HOMA-IR > 3 (*n*, %)	29 (17%)	23 (25%)	0.1184
HCV RNA > 600 000 IU/mL (*n*, %)	94 (55%)	59 (64%)	0.1510
ALT > ULN (*n*, %)	114 (67%)	72 (78%)	0.0488
GGT > ULN (*n*, %)	127 (74%)	60 (65%)	0.1225
Fasting cholesterol mmol/L (median, IQR)	4.41 (3.91–4.96)	3.77 (3.06–4.50)	< 0.0001
Hepatic steatosis (> 5%) (*n*, %)	49 (29%)	45 (49%)	0.0011
METAVIR grade (A2-3) (*n*, %)	85 (50%)	43 (47%)	0.6460
Fibrosis stage (*n*, %)[†]			$P = 0.6085$
F0-F1	129 (76%)	72 (79%)	
F2-F4	40 (24%)	19 (21%)	

[†]Histology was not available in three patients; ALT, alanine aminotransferase; BMI, body mass index; GGT, Gamma-glutamyltransferase; HOMA-IR, Homeostasis Model for Assessment of insulin resistance; IQR, interquartile range.

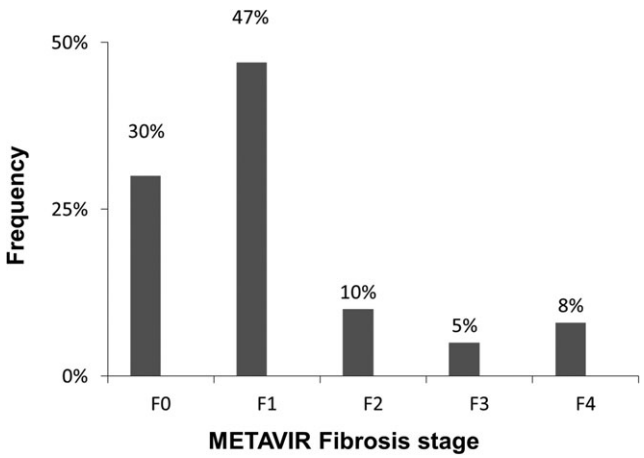

Figure 2 Distribution of METAVIR fibrosis stage for the Asia-region cohort indicating that most patients had no-mild stage fibrosis (METAVIR F0-1 = 77%).

Table 2 Multivariable logistic regression for predictors of significant fibrosis (F2-F4) in Asian region patients

	Odds ratio	Confidence interval	*P*-value
Age	1.065	1.03–1.1	< 0.0001
HOMA-IR ($\log_{10}$)	8.42	2.1–34.3	0.003
Total cholesterol	0.008	< 0.001–0.205	0.0035
METAVIR activity	3.17	2.0–4.9	< 0.0001

Covariates included: age, body mass index (BMI), hepatitis C virus (HCV) RNA ($\log_{10}$ IU/mL), Homeostasis Model for Assessment of insulin resistance (HOMA-IR) ($\log_{10}$ unit), fasting cholesterol ($\log_{10}$ mmol/L), METAVIR necro-inflammatory activity.

of causality for significant disease cannot be implied from cross-sectional observations. Furthermore, there are potential issues with misinterpretation of data based inherent to all studies that rely on post hoc subgroup analysis of clinical trials.[40] However, despite exclusion of patients with missing data and diabetics, the large sample size of nearly 700 patients did allow for correction for multiple testing and confidence in our multivariable analysis. Another limitation of this study was the absence of other anthroprometric measurements of IR or metabolic syndrome. Greater than one-third of our Asian-region cohort and one-half of non-Asians (predominantly Caucasian) had insulin resistance based on HOMA-IR ≥ 2. This likely relates to significant differences in BMI between Asian and non-Asian region patients in this study (Table S1). Due to sample size issues, we did not extend our assessment of insulin resistance and fibrosis to individual Asian region countries that participated in this clinical study, and thus our study findings may not apply to other Asian-region countries. Although a few patients recruited in non-Asian countries may have been of Asian origin, these were unlikely to influence the results significantly. In addition, evaluation of our data according to geographic region removed inherent issues associated with self-reported ethnic background utilized in most non-Asian studies.

Although hyperinsulinemia has been associated with end-stage liver disease due to reduced hepatic insulin extraction, the hepatitis C virus appears to induce insulin resistance and the metabolic syndrome through several complex mechanisms. These include interference of host intracellular insulin signaling pathways,[41] and insulin receptor substrate-1 (IRS-1) degradation mediated through reduced PPAR expression, increased suppressor of cytokine signaling-3 and 7 (SOCS-3 and -7) or activation of mammalian target of rapamycin (mTOR) that may be genotype specific.[2] Induction of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), may also further impair insulin signaling and promote IRS-1 degradation.[42] Certainly, higher HCV RNA levels appear to correlate with IR in CHC

Table 3 Multivariable logistic regression using binary variables for significant fibrosis (F2-F4) in Asian region patients

	Odds ratio	Confidence interval	*P*-value
Age > 40 years	6.50	2.5–16.8	< 0.0001
METAVIR activity A2/3	4.11	2.0–8.4	= 0.0001
Total cholesterol < 130 mg/dL	2.90	1.3–6.6	0.0111
HOMA-IR > 2	2.80	1.4–5.4	0.0022

Covariates included: age > 40 years, hepatitis C virus (HCV) RNA > 600 000 IU/mL, Homeostasis Model for Assessment of insulin resistance (HOMA-IR) > 2, fasting total cholesterol < 130 mg/dL, and METAVIR grade A2/3.
Male gender, HCV genotype, body mass index (BMI) > 25/BMI > 30, past alcohol history, and hepatic steatosis (≥ 5% hepatocytes) were not significant in univariable analyses.

Table 4 Multivariable logistic regression for predictors of significant fibrosis (F2-F4) in non-Asian region patients ($n = 407$)

	Odds ratio	Confidence interval	*P*-value
Age	1.04	1.04–1.1	< 0.0001
HOMA-IR (log_{10})	3.36	1.1–10.2	0.03
Total cholesterol	0.03	0.002–0.45	0.01
METAVIR activity	2.37	1.7–3.4	< 0.0001

Covariates included: age, body mass index (BMI), hepatitis C virus (HCV) RNA (log_{10} IU/mL), Homeostasis Model for Assessment of insulin resistance (HOMA-IR) (log_{10} unit), fasting cholesterol (log_{10} mg/dL), METAVIR necro-inflammatory activity.

patients with genotypes 1 and 4 from non-Asian regions.[10,43] A study from Taiwan evaluating 162 CHC genotype 1 and 2 patients noted an association between higher HCV RNA and HOMA-IR > 2.4 mostly among HCV genotype 1 patients.[5] However, we did not observe a similar correlate, and other studies have also failed to demonstrate an association between HCV RNA levels and HCV genotypes 1–3.[7,29] Interestingly, our parent study also indicated that insulin resistance is reduced following sustained virologic response in HCV genotype 1, but not 2 or 3 infection.[44] These differences for association between viral burden and insulin resistance not only reflect demographic and HOMA-IR threshold variation among study cohorts, but also indicate that other host, and perhaps genotype-specific viral factors, may selectively modulate insulin signaling.

Both experimental and clinical data indicate that IR appears to result in fibrogenesis independently of steatosis. Certainly, insulin may result in hepatic stellate cell activation and increased *in vitro* expression of collagen and connective tissue growth factor.[12] Several studies (in predominantly Caucasian cohorts) have noted an association between IR and fibrosis.[6,13,14,17,29] In contrast to our data indicating an independent association between IR and stage F2-F4 fibrosis in Asian patients, a recent study of 528 CHC genotype 1 or 2 patients from Taiwan did not demonstrate an independent effect of HOMA-IR on advanced stage fibrosis. Instead, elevated fasting blood glucose and BMI were significantly associated with stage F3-F4 disease for HCV genotypes 1 and 2, respectively.[33] However, another smaller study in CHC patients with varying genotypes from Taiwan noted an association between HOMA-IR > 2.5 and low platelet count with advanced stage fibrosis.[32] This likely reflects differences between our population and these study cohorts for genotype, BMI, inclusion of diabetes mellitus patients, and evaluation of a more advanced stage disease as an outcome measure in the Taiwanese studies. However, older age, higher necro-inflammatory activity, lower total cholesterol, but not steatosis were also significantly associated with stage F3-F4 fibrosis in the larger study from Taiwan,[33] and these host factors are comparable to the independent variables selected in our multivariate modeling for both Asian region and non-Asian region patients.

Hepatitis C virus genotype 3 infection is associated with higher fibrosis progression rates,[26] and this effect appears to be independent of steatosis. Furthermore, the influence of steatosis on fibrosis appears significant for genotype non-3 infected patients only. In our study, as expected the prevalence of steatosis was higher among HCV genotype 3 infected patients, although we did not observe any significant differences in fibrosis stage between HCV genotype 2 and 3 infection. Certainly there were observed baseline differences in demographic and host variables between HCV genotype 2 and 3 Asian-region cohorts, in terms of age, steatosis prevalence and total cholesterol, which could potentially impact our results. However, HCV genotype (or steatosis) was not selected as an independent predictor of fibrosis in univariate modeling. Also, there were no baseline differences in insulin resistance or necro-inflammatory activity between the HCV genotype 2 and 3 Asian-region cohorts, but these variables were independently associated with significant fibrosis. Most of the patients enrolled in this study had mild fibrosis and minimal grade steatosis, and may be the main reason for the absence of association between steatosis and fibrosis in our modeling. However, the inclusion of insulin resistance in modeling may also remove the independent effects of steatosis on fibrosis[13] and perhaps indicates a direct pathogenic role for insulin resistance in fibrogenesis.

Hepatitis C virus associates with host lipid metabolism pathways for cellular entry, replication, assembly and secretion.[45] Low lipid levels in CHC have been correlated with steatosis[46,47] and more advanced liver fibrosis.[48,49] There may have been a possible covariate effect between necro-inflammation and low cholesterol as independent predictors for significant fibrosis in our study, but we did not observe any significant association between ALT or histologic activity and total cholesterol. Perhaps genotype-specific interference with very-low-density lipoprotein (VLDL) assembly and secretion may result in the lower total cholesterol levels seen in HCV genotype 3 infection, but the biological pathways linking host-viral effects on lipid metabolism and fibrogenesis have yet to be clearly defined.

In summary, this study indicates that insulin resistance is present in a significant proportion of Asian region HCV genotype 2 and 3 infected patients, and along with age, hepatic inflammation and low cholesterol, may be associated with significant hepatic fibrosis. A possible implication of our observations is that perhaps lifestyle and therapeutic measures to improve insulin sensitivity may result in reduced disease progression in HCV genotype 2 and 3 infected patients, along with reducing associated cardiovascular and metabolic syndrome risk factors. However, prospective studies to confirm these observations, and evaluate the effects of modulation of host metabolic risk factors on slowing disease progression in CHC infection, require further consideration.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics for Asian region patients *vs* Non-Asian region patients chronically infected with genotype 2 or 3 hepatitis C virus (HCV).

Table S2. Multivariate logistic model for insulin resistance in Asian-region patients.

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