Int J Mol Epidemiol Genet 2018;9(2):13-19
www.ijmeg.org /ISSN:1948-1756/IJMEG0073736

Original Article

Hepatic steatosis in chronic hepatitis B: a study of metabolic and genetic factors

Michael O Baclig[1], Karen G Reyes[1], Veni R Liles[1], Cynthia A Mapua[1], Mark Pierre S Dimamay[1], Juliet Gopez-Cervantes[2]

[1]Research and Biotechnology, [2]Liver Disease and Transplant Center, St. Luke's Medical Center, 279 E. Rodriguez Sr. Blvd., Quezon 1112, Philippines

Received January 31, 2018; Accepted March 19, 2018; Epub April 5, 2018; Published April 15, 2018

Abstract: Hepatic steatosis is a common finding in liver biopsy and may co-exist with chronic hepatitis B (CHB) infection. The aims of this study were to determine the prevalence of steatosis in CHB patients among Filipinos; determine the factors related to the presence of steatosis among individuals with and without CHB infection; and to investigate the possible association between steatosis and polymorphism in interleukin 28B (IL28B) gene. The presence of steatosis was correlated with clinical, biochemical and histological parameters. Peripheral blood samples of CHB patients with steatosis, CHB patients without steatosis and normal controls were genotyped for IL28B rs8099917 T>G using the TaqMan assay. Of the 46 CHB patients, 41% (19/46) had steatosis. Body mass index (BMI), fasting blood sugar (FBS), lipid profile and alanine transaminase levels were observed to be significantly different between CHB patients with steatosis and normal controls. The serum FBS of CHB patients with steatosis was significantly higher than patients without steatosis. High density lipoprotein cholesterol of patients without steatosis was significantly higher than patients with steatosis. Although not statistically significant, BMI, triglycerides, low density lipoprotein cholesterol and histology activity index in CHB patients with steatosis were found to be higher than those without steatosis. There was no significant association between the stage of fibrosis and severity of steatosis. In conclusion, the prevalence of hepatic steatosis among Filipino patients with CHB is 41%. Steatosis in CHB patients was associated with metabolic factors such as diabetes and dyslipidemia. IL28B rs8099917 T>G polymorphism is not associated with steatosis.

Keywords: Chronic hepatitis B, hepatic steatosis, interleukin 28B rs8099917, interferon λ, metabolic factors, polymorphism

Introduction

Hepatic steatosis is a common histopathological feature of chronic hepatitis B (CHB) and is found in approximately 14% to 70% of cases. Hepatic steatosis is characterized by the deposition of lipid droplets in the hepatocytes that exceed 5% of the gross weight of the liver [1-3]. Several factors are capable of inducing hepatic steatosis such as obesity, dyslipidemia, non-alcoholic fatty liver disease (NAFLD), diabetes and insulin resistance [4, 5].

Interleukin 28B (IL28B) encodes a protein known as interferon λ3 which include IL29 and IL28A. Previous studies have demonstrated that genetic polymorphism in IL28B strongly predict sustained virologic response to pegylated interferon-α and ribavirin treatment in patients with chronic hepatitis C (CHC) [6-9]. Other studies have demonstrated that IL28B rs12979860 C>T and rs8099917 T>G polymorphisms were associated with insulin resistance, steatosis and fibrosis among patients with CHC [10-12]. In another study, it has been shown that the presence of IL28B rs12979860 CC genotype and patatin-like phospholipase domain-containing protein 3 (PNPLA3) rs738409 GG genotype have increased the risk of steatohepatitis in patients with NAFLD [13]. Recently, it has been reported that IL28B rs12979860 CC genotype may be negatively associated with hepatic steatosis in Asian CHC patients [5]. Whether IL28B rs8099917 polymorphism is associated with an increased risk of hepatic steatosis in CHB patients is largely unknown.

Table 1. Clinical and laboratory characteristics of CHB patients and normal controls

	CHB without steatosis N = 27	CHB with steatosis N = 19	Normal controls N = 23	*P* value
	Mean (SD)	Mean (SD)	Mean (SD)	
Age	41.7 (13.1)	47.5 (11.3)	28.7 (8.6)	<0.001
BMI (kg/m^2)	25.1 (5.7)	26.4 (3.9)	22.0 (1.8)	0.005
FBS (mg/dl)	90.9 (11.4)	106.1 (28.9)	86.9 (9.2)	0.002
Cholesterol (mg/dl)	201.9 (46.0)	206.1 (38.4)	200.0 (41.5)	NS
Triglycerides (mg/dl)	108.8 (62.6)	134.9 (45.3)	68.9 (41.5)	<0.001
HDL-C (mg/dl)	49.5 (17.2)	37.5 (9.3)	60.0 (17.8)	<0.001
LDL-C (mg/dl)	124.2 (37.5)	138.1 (34.2)	121.5 (35.0)	NS
VLDL-C (mg/dl)	21.6 (12.6)	26.9 (9.0)	13.9 (8.3)	0.001
Non-HDL-C (mg/dl)	152.3 (46.4)	168.6 (39.6)	140.0 (41.3)	NS
AST (IU/L)	42.2 (46.9)	46.4 (34.5)	21.2 (8.5)	0.054
ALT (IU/L)	83.6 (126.3)	75.4 (43.9)	42.3 (15.0)	NS

Abbreviations: BMI, body mass index; FBS, fasting blood sugar; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low density lipoprotein; Non-HDL-C, non-high density lipoprotein cholesterol (total cholesterol minus HDL-C); AST, aspartate transaminase; ALT, alanine transaminase; IL28B, interleukin 28B; SD, standard deviation; NS, not significant.

Table 2. Histological characteristics of CHB patients

	CHB without steatosis N (%)	CHB with steatosis N (%)	*P value*
Metavir			NS
A1-A2	23 (85.2)	14 (77.8)	
A3-A4	4 (14.8)	4 (22.2)	
Fibrosis			NS
F0-F2	25 (92.6)	18 (100.0)	
F3-F4	2 (7.4)	0 (0.0)	

Abbreviations: CHB, chronic hepatitis B; NS, not significant.

The aims of this study were to determine the prevalence of hepatic steatosis in CHB patients among Filipinos; determine the factors related to the presence of hepatic steatosis and to investigate the possible association between hepatic steatosis and polymorphism in IL28B gene. A clear understanding of the prevalence of hepatic steatosis in CHB could provide useful information for the improvement of care of patients at risk for developing advanced liver damage.

Materials and methods

Patients and samples

A total of 46 CHB patients and 23 normal controls aged 21 to 67 years old from January 2012 to December 2013 were consecutively enrolled in this study. Peripheral blood samples were genotyped for IL-28B rs8099917 T>G using the TaqMan assay. The inclusion criteria considered were the following: HBsAg positive by serology, HBV DNA positive by PCR and a liver biopsy consistent with the diagnosis of CHB. Patients co-infected with hepatitis C virus were excluded from the study. The control group presented with normal body mass index (BMI), normal ultrasound and normal blood chemistry including but are not limited to serum fasting blood sugar (FBS), total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), non-HDL cholesterol (total cholesterol minus HDL-C), aspartate transaminase (AST) and alanine transaminase (ALT). Written informed consent was obtained from each participant. The study was approved by the St. Luke's Institutional Ethics Review Committee.

Nucleic acid extraction

Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) or the Taqman sample-to-SNP kit (Applied Biosystems, Foster City, CA, USA) according to manufacturer's protocol. The DNA concentration and purity were measured using the NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). All samples were stored at -80°C prior to testing.

Real-time PCR

Genotyping for IL28B rs8099917 T>G was carried out using the TaqMan SNP assay ID C_11710096_10 (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed on the Rotor-Gene Q instrument (Qiagen, Hilden, Germany) under the following conditions: hold at 95°C for 20 seconds, 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Allelic discrimination was performed using probes labeled with the fluorescent dyes VIC

Table 3. Comparison of clinical and laboratory characteristics of CHB patients without steatosis and normal controls

	CHB without steatosis	Normal controls	*P* value
	Mean (SD)	Mean (SD)	
Age	41.7 (13.1)	28.7 (8.6)	<0.001
BMI (kg/m^2)	25.1 (5.7)	22.0 (1.8)	0.019
FBS (mg/dl)	90.9 (11.4)	86.9 (9.2)	NS
Cholesterol (mg/dl)	201.9 (46.0)	200.0 (41.5)	NS
Triglycerides (mg/dl)	108.8 (62.6)	68.9 (41.5)	0.012
HDL-C (mg/dl)	49.5 (17.2)	60.0 (17.8)	0.041
LDL-C (mg/dl)	124.2 (37.5)	121.5 (35.0)	NS
VLDL-C (mg/dl)	21.6 (12.6)	13.9 (8.3)	0.016
Non-HDL-C (mg/dl)	152.3 (46.4)	140.0 (41.3)	NS
AST (IU/L)	42.2 (46.9)	21.2 (8.5)	0.049
ALT (IU/L)	83.6 (126.3)	42.3 (15.0)	NS

Independent t-test was performed to determine the differences between the two groups. BMI, body mass index; FBS, fasting blood sugar; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low density lipoprotein; Non-HDL-C, non-high density lipoprotein cholesterol (total cholesterol minus HDL-C); AST, aspartate transaminase; ALT, alanine transaminase; NS, not significant.

Table 4. Comparison of clinical and laboratory characteristics of CHB patients with steatosis and normal controls

	CHB with steatosis	Normal controls	*P* value
	Mean (SD)	Mean (SD)	
Age	47.5 (11.3)	28.7 (8.6)	<0.001
BMI (kg/m^2)	26.4 (3.9)	22.0 (1.8)	<0.001
FBS (mg/dl)	106.1 (28.9)	86.9 (9.2)	0.004
Cholesterol (mg/dl)	206.1 (38.4)	200.0 (41.5)	NS
Triglycerides (mg/dl)	134.9 (45.3)	68.9 (41.5)	<0.001
HDL-C (mg/dl)	37.5 (9.3)	60.0 (17.8)	<0.001
LDL-C (mg/dl)	138.1 (34.2)	121.5 (35.0)	NS
VLDL-C (mg/dl)	26.9 (9.0)	13.9 (8.3)	<0.001
Non-HDL-C (mg/dl)	168.6 (39.6)	140.0 (41.3)	0.029
AST (IU/L)	46.4 (34.5)	21.2 (8.5)	0.003
ALT (IU/L)	75.4 (43.9)	42.3 (15.0)	0.002

Independent t-test was performed to determine the differences between the two groups. BMI, body mass index; FBS, fasting blood sugar; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low density lipoprotein; Non-HDL-C, non-high density lipoprotein cholesterol (total cholesterol minus HDL-C); AST, aspartate transaminase; ALT, alanine transaminase; NS, not significant.

and FAM. IL28B rs8099917 is bi-allelic with 3 possible genotypes as follows: TT, homozygous wild-type; TG, heterozygous and GG, homozygous variant. IL28B genotyping was undertaken blinded to clinical, biochemical and histological data. The IL28B rs8099917 T>G single nucleotide polymorphism (SNP) was chosen because this variant is common among Asians [14].

Histological evaluation

The liver biopsies obtained in 46 CHB patients were assessed using the Metavir and Knodell Histology Activity Index (HAI) scoring system. Steatosis was assessed as the percentage of hepatocytes containing fat droplets. Grading of steatosis was categorized as absent (0%), minimal (less than 5%), mild (5% to 33%), moderate (34% to 66%) and severe (more than 66%).

Statistical analysis

Continuous variables were expressed as mean ± SD and compared using Student's t-test or ANOVA for multiple comparisons. Categorical variables were expressed as percentages and compared using the Chi-square test or Fisher's exact test. All analyses were conducted using statistical program for social sciences statistical software. Statistical significance was determined at $P \leq 0.05$.

Results

Patient characteristics and laboratory data

The baseline characteristics of the 46 CHB patients are summarized in **Table 1**. There were 31 men (67%) and 15 women (33%). Among CHB patients without steatosis, the mean age was 41.7 ± 13.1 while those with steatosis the mean age was 47.5 ± 11.3. Among CHB patients with steatosis, the mean BMI was 26.4 ± 3.9 kg/m^2, serum FBS 106.1 ± 28.9 mg/dl, while the mean triglyceride, HDL-C and LDL-C levels were 134.9 ± 45.3 mg/dl, 37.5 ± 9.3 mg/dl and 138.1 ± 34.2 mg/dl, respectively.

Of the 46 CHB patients, 41% (19/46) had steatosis. Mild steatosis was observed in 66.7% (12/18), moderate steatosis in 22.2% (4/18) and severe steatosis in 11.1% (2/18).

Table 5. Comparison of clinical and laboratory characteristics of CHB patients without steatosis and CHB patients with steatosis

	CHB without steatosis	CHB with steatosis	*P* value
	Mean (SD)	Mean (SD)	
Age	41.7 (13.1)	47.5 (11.3)	NS
BMI (kg/m^2)	25.1 (5.7)	26.4 (3.9)	NS
FBS (mg/dl)	90.9 (11.4)	106.1 (28.9)	0.017
Cholesterol (mg/dl)	201.9 (46.0)	206.1 (38.4)	NS
Triglycerides (mg/dl)	108.8 (62.6)	134.9 (45.3)	NS
HDL-C (mg/dl)	49.5 (17.2)	37.5 (9.3)	0.008
LDL-C (mg/dl)	124.2 (37.5)	138.1 (34.2)	NS
VLDL-C (mg/dl)	21.6 (12.6)	26.9 (9.0)	NS
Non-HDL-C (mg/dl)	152.3 (46.4)	168.6 (39.6)	NS
AST (IU/L)	42.2 (46.9)	46.4 (34.5)	NS
ALT (IU/L)	83.6 (126.3)	75.4 (43.9)	NS
HAI	4.9 (3.8)	7.1 (4.25)	NS

Independent t-test was performed to determine the differences between the two groups. BMI, body mass index; FBS, fasting blood sugar; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low density lipoprotein; Non-HDL-C, non-high density lipoprotein cholesterol (total cholesterol minus HDL-C); AST, aspartate transaminase; ALT, alanine transaminase; HAI, histology activity index; NS, not significant.

Table 6. Distribution of IL28B genotypes in CHB patients and normal controls

	CHB without steatosis N = 27	CHB with steatosis N = 19	Normal controls N = 23
IL28B rs8099917			
T allele	0.72	0.89	0.76
G allele	0.28	0.11	0.24
IL28B rs8099917			
TT genotype (%)	13 (48.1)	15 (78.9)	14 (60.9)
TG genotype (%)	13 (48.1)	4 (21.1)	7 (30.4)
GG genotype (%)	1 (3.7)	0 (0)	2 (8.7)

Abbreviations: CHB, chronic hepatitis B; IL28B, interleukin 28B; T, frequency of the major allele; G, frequency of the minor allele; TT, homozygous wild-type; TG, heterozygous; GG, homozygous variant.

The Metavir and HAI scores are shown in **Table 2**. There was no significant association between the stage of fibrosis and severity of steatosis.

Age ($P \leq 0.001$), BMI ($P = 0.019$), triglyceride ($P = 0.012$), HDL-C ($P = 0.041$) and VLDL-C ($P = 0.016$) levels were found to be significantly different between CHB patients without steatosis and normal controls (**Table 3**). On the other hand, age ($P \leq 0.001$), BMI, ($P \leq 0.001$), FBS ($P = 0.004$), triglyceride ($P \leq 0.001$), HDL-C ($P \leq 0.001$), VLDL-C ($P \leq 0.001$), non-HDL-C ($P = 0.029$), AST ($P = 0.003$) and ALT ($P = 0.002$) levels were observed to be significantly different between CHB patients with steatosis and normal controls (**Table 4**).

Serum FBS of CHB patients with steatosis was found to be significantly higher than patients without steatosis ($P = 0.017$). HDL-C of patients without steatosis was observed to be higher than patients with steatosis ($P = 0.008$). Although not statistically significant, BMI, triglyceride levels, LDL-C and HAI in CHB patients with steatosis were found to be higher than those without steatosis (**Table 5**).

Distribution of IL28B genotypes

The IL28B rs8099917 T>G was successfully genotyped in 100% of the samples. The distribution of IL28B genotypes in CHB patients and normal controls are shown in **Table 6**. Among CHB patients with steatosis, the allelic frequencies in Hardy Weinberg equilibrium were 0.89 for the T allele and 0.11 for the G allele. No significant differences were found in the allelic and genotypic frequencies of IL28B rs8099917 polymorphism between CHB patients and normal controls. There was no significant association between IL28B rs8099917 T>G polymorphism and hepatic steatosis in this study.

Discussion

The prevalence of hepatic steatosis in CHC and the mechanisms associated with steatosis have been well described. However, studies for hepatic steatosis and CHB are limited [2, 15, 16]. Our data provide evidence that hepatic steatosis is present in 41% of Filipino patients with CHB. The presence of steatosis is associated with FBS and HDL-C levels. Consistent with a previous report, FBS level was significantly higher among CHB patients with steatosis (106.1 ± 28.9 mg/dl) as compared with CHB patients without steatosis (90.9 ± 11.4 mg/dl). The present study is also in keeping with a previous report indicating that hepatic steatosis in CHB is not associated with the severity of fibrosis [3, 15, 17, 18].

There are many reports of genetic polymorphisms involved in lipid metabolism. Among these polymorphisms, PNPLA3 and transmembrane 6 superfamily member 2 (TM6SF2) have gained the most attention [12]. Here, we set out to investigate the possible association between IL28B rs8099917 T>G polymorphism and the presence of steatosis. Our result shows that there is no association between genetic polymorphism in IL28B rs8099917 and hepatic steatosis in CHB patients. In contrast, a meta-analysis showed that the IL28B rs8099917 TT genotype was significantly associated with a lower risk of steatosis in CHC [11]. Association analysis of haplotypes consisting of several SNPs such as rs12979860, rs8099917, rs8105790 and rs10853728 could provide better coverage value of the IL28B genotypes over a single variant analysis [19, 20].

The mechanism of steatosis induced by CHB has remained elusive. A previous study has shown that lipid accumulation may be due to the inhibition of secretion of apolipoprotein B by hepatitis B virus (HBV) X protein by enhancing the expression of N-acetylglucosaminyltransferase-III [21, 22]. In another study, it has been shown that increased HBV X protein expression via transcriptional activation of sterol regulatory element binding protein 1 and peroxisome proliferator-activated receptor increases lipid accumulation [23]. Recently, it has been demonstrated that HBV X protein expression is associated with increased oxidative stress and damage [24]. In another study, it has been shown that TM6SF2 E167K substitution promotes steatosis and lipid abnormalities by altering TM6SF2 and microsomal triglyceride transfer protein expression [25].

One of the strengths of this study is that liver biopsy was used to diagnose hepatic steatosis. In previous studies, hepatic steatosis was based on non-invasive imaging modalities such as ultrasonography [26, 27]. To the best of our knowledge, this study is the first to have reported the prevalence of hepatic steatosis in CHB among Filipinos. However, our study is not without limitations. First, we were unable to evaluate the potential impact of alcohol consumption on hepatic steatosis. Second, IL28B rs12979860 genotyping was not done in this study. Recently, it has been reported that IL28B rs12979860 genotyping is highly informative in staging fibrosis in CHB, CHC and NAFLD patients [28]. Third, the relatively small sample size could have missed an association between hepatic steatosis and IL28B rs8099917 T>G polymorphism. Thus, further prospective studies are needed to validate our findings.

In conclusion, the prevalence of hepatic steatosis among Filipino patients with CHB is 41%. Hepatic steatosis in CHB patients was associated with metabolic factors such as dyslipidemia and diabetes. IL28B rs8099917 T>G polymorphism is not associated with hepatic steatosis.

Acknowledgements

This work was supported by a research grant (Project No. 12-002c) to the St. Luke's Liver Diseases Study Group through the Research and Biotechnology, St. Luke's Medical Center-Quezon City.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Michael O Baclig, Research and Biotechnology, St. Luke's Medical Center, 279 E. Rodriguez Sr. Blvd., Quezon 1112, Philippines. Tel: +63 (2) 7230101 local 4203; E-mail: mobaclig@stluke.com.ph

References

[1] Gordon A, McLean CA, Pedersen JS, Bailey MJ, Roberts SK. Hepatic steatosis in chronic hepatitis B and C: predictors, distribution and effect on fibrosis. J Hepatol 2005; 43: 38-44.

[2] Pais R, Rusu E, Zilisteanu D, Circiumaru A, Micu L, Voiculescu M, Poynard T, Ratziu V. Prevalence of steatosis and insulin resistance in patients with chronic hepatitis B compared with chronic hepatitis C and non-alcoholic fatty liver disease. Eur J Intern Med 2015; 26: 30-36.

[3] Thomopoulos KC, Arvaniti V, Tsamantas AC, Dimitropoulou D, Gogos CA, Siagris D, Theocharis GJ, Labropoulou-Karatza C. Prevalence of liver steatosis in patients with chronic hepatitis B: a study of associated factors and of relationship with fibrosis. Eur J Gastroenterol Hepatol 2006; 18: 233-237.

[4] Bondini S, Kallman J, Wheeler A, Prakash S, Gramlich T, Jondle DM, Younossi ZM. Impact of non-alcoholic fatty liver disease on chronic hepatitis B. Liver Int 2007; 27: 607-611.

[5] Huang CM, Chang KC, Hung CH, Chiu KW, Lu SN, Wang JH, Chen CH, Kee KM, Kuo YH, Tsai MC, Tseng PL, Lin MT, Wu CK, Hu TH, Cho CL, Yen YH. Impact of PNPLA3 and IFNL3 polymorphisms on hepatic steatosis in Asian patients with chronic hepatitis C. PLoS One 2017; 12: e0182204.

[6] Uygun A, Ozturk K, Demirci H, Oztuna A, Eren F, Kozan S, Yilmaz Y, Kurt O, Turker T, Vatansever S, Alper E, Unsal B. The association of nonalcoholic fatty liver disease with genetic polymorphisms: a multicenter study. Eur J Gastroenterol Hepatol 2017; 29: 441-447.

[7] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009; 461: 399-401.

[8] Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009; 41: 1100-1104.

[9] Thompson AJ, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, Urban T, Afdhal NH, Jacobson IM, Esteban R, Poordad F, Lawitz EJ, McCone J, Shiffman ML, Galler GW, Lee WM, Reindollar R, King JW, Kwo PY, Ghalib RH, Freilich B, Nyberg LM, Zeuzem S, Poynard T, Vock DM, Pieper KS, Patel K, Tillmann HL, Noviello S, Koury K, Pedicone LD, Brass CA, Albrecht JK, Goldstein DB, McHutchison JG. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. Gastroenterology 2010; 139: 120-9, e18.

[10] Agúndez JA, García-Martin E, Maestro ML, Cuenca F, Martínez C, Ortega L, Carballo M, Vidaurreta M, Agreda M, Díaz-Zelaya G, Suárez A, Díaz-Rubio M, Ladero JM. Relation of IL28B gene polymorphism with biochemical and histological features in hepatitis C virus-induced liver disease. PLoS One 2012; 7: e37998.

[11] Sato M, Kondo M, Tateishi R, Fujiwara N, Kato N, Yoshida H, Taguri M, Koike K. Impact of IL28B genetic variation on HCV-induced liver fibrosis, inflammation and steatosis: a meta-analysis. PLoS One 2014; 9: e91822.

[12] Miyaaki H, Nakao K. Significance of genetic polymorphisms in patients with non-alcoholic fatty liver disease. Clin J Gastroenterol 2017; 10: 201-207.

[13] Petta S, Grimaudo S, Cammà C, Cabibi D, Di Marco V, Licata G, Pipitone RM, Craxì A. IL28B and PNPLA3 polymorphisms affect histological liver damage in patients with non-alcoholic fatty liver disease. J Hepatol 2012; 56: 1356-1362.

[14] Mah YH, Liu CH, Chen CL, Tseng TC, Liu CJ, Chen PJ, Chen DS, Kao JH. Prevalence and clinical implications of IL28B genotypes in Taiwanese patients with chronic hepatitis C. J Formos Med Assoc 2016; 115: 953-960.

[15] Peng D, Han Y, Ding H, Wei L. Hepatic steatosis in chronic hepatitis B patients is associated with metabolic factors more than viral factors. J Gastroenterol Hepatol 2008; 23: 1082-1088.

[16] Wang MM, Wang GS, Shen F, Chen GY, Pan Q, Fan JG. Hepatic steatosis is highly prevalent in hepatitis B patients and negatively associated with virological factors. Dig Dis Sci 2014; 59: 2571-2579.

[17] Shi JP, Fan JG, Wu R, Gao XQ, Zhang L, Wang H, Farrell GC. Prevalence and risk factors of hepatic steatosis and its impact on liver injury in Chinese patients with chronic hepatitis B infection. J Gastroenterol Hepatol 2008; 23: 1419-1425.

[18] Machado MV, Oliveira AG, Cortez-Pinto H. Hepatic steatosis in hepatitis B virus infected patients: Meta-analysis of risk factors and comparison with hepatitis C infected patients. J Gastroenterol Hepatol 2011; 26: 1361-1367.

[19] Stram DO. Multi-SNP haplotype analysis methods for association analysis. Methods Mol Biol 2017; 1666: 485-504.

[20] Lee C, Lin CH, Huang YH, Huo TI, Su CW, Hou MC, Huang HC, Lee KC, Chan CC, Lin MW, Lin HC, Lee SD. IL28B polymorphism correlates with active hepatitis in patients with HBeAg-negative chronic hepatitis B. PLoS One 2013; 8: e58071.

[21] Lim CT, Kumar R. Hepatitis B and concomitant hepatic steatosis. Ann Transl Med 2017; 5: 38.

[22] Kang SK, Chung TW, Lee JY, Lee YC, Morton RE, Kim CH. The hepatitis B virus X protein inhibits secretion of apolipoprotein B by enhancing the expression of N-acetylglucosaminyltransferase III. J Biol Chem 2004; 279: 28106-28112.

[23] Kim KH, Shin HJ, Kim K, Choi HM, Rhee SH, Moon HB, Kim HH, Yang US, Yu DY, Cheong J. Hepatitis B virus X protein induces hepatic steatosis via transcriptional activation of SREBP1 and PPARgamma. Gastroenterology 2007; 132: 1955-1967.

[24] Ren JH, Chen X, Zhou L, Tao NN, Zhou HZ, Liu B, Li WY, Huang AL, Chen J. Protective role of sirtuin3 (SIRT3) in oxidative stress mediated by Hepatitis B virus X protein expression. PLoS One 2016; 11: e0150961.

[25] Eslam M, Mangia A, Berg T. Diverse impacts of the rs58542926 E167K variant in TM6SF2 on

viral and metabolic liver disease phenotypes. Hepatology 2016; 64: 34-46.

[26] Cheng YL, Wang YJ, Kao WY. Inverse association between hepatitis B virus infection and fatty liver disease: a large-scale study in populations seeking for check-up. PLoS One 2013; 8: e72049.

[27] Wong VW, Wong GL, Chu WC, Chim AM, Ong A, Yeung DK, Yiu KK, Chu SH, Chan HY, Woo J, Chan FK, Chan HL. Hepatitis B virus infection and fatty liver in the general population. J Hepatol 2012; 56: 533-540.

[28] Eslam M, Hashem AM, Leung R, Romero-Gomez M, Berg T, Dore GJ, Chan HL, Irving WL, Sheridan D, Abate ML, Adams LA Mangia A, Weltman M, Bugianesi E, Spengler U, Shaker O, Fischer J, Mollison L, Cheng W, Powell E, Nattermann J, Riordan S, McLeod D, Armstrong NJ, Douglas MW, Liddle C, Booth DR, George J, Ahlenstiel G; International Hepatitis C Genetics Consortium (IHCGC). Interferon-λ rs12979860 genotype and liver fibrosis in viral and non-viral chronic liver disease. Nat Commun 2015; 6: 6422.