RESEARCH ARTICLE

Editorial Process: Submission:01/19/2018 Acceptance:07/23/2018

The Association of PNPLA3, COX-2 and DHCR7 Polymorphisms with Advanced Liver Fibrosis in Patients with HCV Mono-Infection and HCV/HIV Co-Infection

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Abstract

There is increasing evidence that host genetic variations may influence the natural history of chronic hepatitis C virus (HCV) infection. The aim of this study was to determine the association between single nucleotide polymorphisms (SNPs) of PNPLA3 (rs738409), COX-2 (rs689465) and DHCR7 (rs12785878) and advanced liver fibrosis in Thai patients. A total of 220 patients with HCV mono-infection, 200 patients with HCV/HIV co-infection and 200 healthy controls were enrolled. The SNPs were detected by allelic discrimination using real-time PCR with TaqMan probes. Liver stiffness measurement (LSM) was assessed by transient elastography. Our results showed that the distribution of the studied SNPs were not significantly different between the HCV mono- and co-infected groups. The frequencies AG and GG genotypes of rs689465 and GG genotype of rs12785878 were less commonly found in the HCV mono- and co-infected groups compare with healthy controls ($P<0.01$). Among patients with HCV infection, older age, HIV co-infection, GG genotype of rs738409 and GG genotype of rs689465 were independently associated with advanced liver fibrosis (LSM≥9.5 kPa) in multivariate analysis. Moreover, the percentage of patients with advanced liver fibrosis increased significantly along with the accumulated numbers of these risk genotypes. In conclusion, PNPLA3 (rs738409) and COX-2 (rs689465) polymorphisms were associated with advanced liver fibrosis in patients with HCV mono- and co-infection, suggesting that these variants might play an important role in progressive liver fibrosis in these patients.

Keywords: Polymorphisms- HCV- HIV- fibrosis- cirrhosis

Asian Pac J Cancer Prev, **19 (8)**, 2191-2197

Introduction

Hepatitis C virus (HCV) infection is an important etiological factor of chronic liver disease worldwide, with an estimated of more than 170 million people currently infected with the virus (Lemoine and Thursz, 2014). Most infected individuals develop into chronic hepatitis, which could progress to cirrhosis and the occurrence of hepatocellular carcinoma (HCC) (Intaraprasong et al., 2016). Various viral, host and environmental factors have been influenced the chronicity and disease progression, including male sex, time of HCV infection, obesity, diabetes, alcohol consumption and co-infection with human immunodeficiency (HIV) (Hajarizadeh et al., 2013). In fact, HIV infection could modify the natural history of chronic HCV infection in co-infected patients, with a higher likelihood of fibrosis progression and cirrhosis. For example, our previous data showed that approximately 40% and 25% of HCV/HIV co-infected and HCV mono-infected patients had advanced liver fibrosis (Avihingsanon et al., 2014).

Host genetic variations have been implicated to influence the natural history of chronic HCV infection. Recent studies have shown that several single nucleotide polymorphisms (SNPs) are related to HCV infection susceptibility and disease progression (Matsuura and Tanaka, 2017). In this setting, Patatin-Like phospholipase domain containing protein 3 (PNPLA3) is involved in lipid storage and its activity has been reported in hydrolysis of triglyceride in the liver (Trepo et al., 2016). The genetic variations of PNPLA3 (rs738409) encoding isoleucine to methionine has been identified to be associated with liver steatosis and fibrosis in patients with HCV infection (Trepo et al., 2016). Moreover, several potent mediators of inflammation are thought to be involved in the process of persistent liver injury and progression of liver fibrosis. In this setting, the polymorphism of cyclooxygenase-2 (COX-2, rs689465) has been linked to pro-inflammatory metabolism, severity of liver fibrosis and HCC development (Miyashita et al., 2012; Bu and

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Zhao, 2013). In addition, there have increasing data that gene-associated with vitamin D metabolism plays an important role in severity of liver fibrosis (Grunhage et al., 2012). For example, a variant of 7-Dehydrocholesterol reductase (DHCR7) (rs12785878), the rate-limiting enzyme of vitamin D metabolism, is associated with lower vitamin D levels and promotes advance fibrosis in patients with chronic HCV infection (Petta et al., 2013). Previous data of our group have also suggested vitamin D deficiency was common in chronic HCV infection and might be associated with progressive liver fibrosis (Avihingsanon et al., 2014). Thus, the aim of this study was to determine the correlation of PNPLA3 (rs738409), COX-2 (rs689465) and DHCR7 (rs12785878) polymorphisms with severity of liver fibrosis in Thai individuals.

Materials and Methods

Patients

From 2011 to 2014, 220 patients with chronic HCV mono-infection and 200 patients with HCV/HIV co-infection were enrolled from King Chulalongkorn Memorial Hospital (Bangkok, Thailand) and the HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT, Bangkok, Thailand), respectively. In addition, 200 healthy controls were recruited from The Thai Red Cross Society (Bangkok, Thailand) at the same period. HCV mono-infection was defined by anti-HCV and HCV RNA positive without evidence of HIV infection, while HCV/HIV co-infection was defined by anti-HCV, HCV RNA and anti-HIV positive. Patients with hepatitis B antigen surface antigen (HBsAg) positive or evidence of HCC were excluded.

All participants signed an informed consent before recruited into the study. The research protocol was approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University (IRB 412/57 and 583/57).

Genotyping of the SNPs

Peripheral blood mononuclear cells (PBMCs) were stored at -80 ^{o}C until SNP genotyping was performed. Human genomic DNA was extracted and purified by phenol chloroform isoamyl alcohol method. Positive control was obtained from HeLa and HepG2 cell line.

PNPLA3 was performed with the forward primer 5′-TACCACGCCTCTGAAGGAAG-3′, the reverse primer 5′-CCCTGCTCACTTGGAGAAAG-3′ (Falleti et al., 2011) and COX-2 was obtained with the forward primer 5′- GAGCACTACCCATGATAGATGTTAAACA-3′, the reverse primer 5′ - TCTCGTTTTGGAACATAGT TGGATGAG-3′ (Miyashita et al., 2012). PCR amplification was containing 0.5 µM forward and reverse primer, 0.5 µM deoxynucleotidetriphosphate (dNTP), 0.6 units of dreamTaq DNA polymerase (thermo scientific, USA) and 100-500 ng of DNA sample in total volume of 25 µl. PCR condition was consisted with denaturation at 95 ^{o}C for 30 s, annealing at 58°C for 30 s and elongation at 72 ^{o}C for 30 s in total 40 cycles. Genotyping was verified by TaqMan probe, PNPLA3 rs738409 (Assay ID: C_7241_10), COX-2 rs689465 (Assay ID: C_2517146_10) and DHCR7 rs12785878 (Assay ID: C_32063037_10) that classified genotype by allelic discrimination method (Applied Biosystems, Foster City, CA) (Zhang et al., 2012). PCR reaction for genotyping was performed with Perfect Taq Master Mix (5 PRIME, Darmstadt, Germany) in total volume of 10 µl as recommended by the manufacturer's instruction. The PCR conditions were followed by holding stage at 95°C for 10 min, denaturation at 92°C for 15 s and annealing/extension at 60°C for 1 min in total 40 cycles. The fluorescence signals (VIC and FAM) that specific to each SNPs were detected and reported by the ABI Step One Plus real-time PCR system (Applied Biosystems).

Liver stiffness measurement

Liver stiffness measurement (LSM) was obtained from each patient with HCV mono-infection and HCV/HIV co-infection after fasting for at least 2 hours by transient elastography (FibroScan, Echosens, Paris, France). Results were recorded in kilopascals (kPa) as the median value of all measurements. The procedure was based on at least 10 validated measurements: the success rate (ratio between numbers of validated and total measurements) was over 60% and interquartile range was less than 30% (Castera et al., 2012). Liver fibrosis stages were defined according to LSM: F0-F1 (<7.1 kPa), F2 (7.1-9.4 kPa), F3 (9.5-14.0) and F4 (>14.0 kPa) (Avihingsanon et al., 2014).

Statistical analysis

Genotyping of the SNPs was reported in allelic discrimination plot. Data were presented as Mean ± standard deviation (SD) and categorical variables as frequency and percentage as appropriate. Frequencies (%) of genotype distribution in study population data were compared to the control groups using online GraphPad Software (http://www.graphpad.com). Moreover, the correlation of the studied SNPs with advanced liver fibrosis was calculated by using a binary variable and the univariate odds ratios (OR) in MedCal Software (http://www.medcalc.org). The results were considered as significant when $P<0.05$ (two-tailed) and frequencies by chi-square test using SPSS software version 22.0.

Results

Baseline characteristics of the participants

The characteristics of all participants are shown in Table 1. The mean age of the healthy control group was significantly higher compared with the other groups ($P<0.001$). However, there was no significant difference in mean age among the HCV mono-infected and HCV/HIV co-infected groups. HCV/HIV co-infected patients had higher proportion of male gender compared the other groups ($P<0.001$). In addition, HCV/HIV co-infected patients had significantly higher mean LSM compared with HCV mono-infected patients ($P<0.001$). There was no difference between HCV mono-infected and HCV/HIV co-infected patients in terms of serum alanine aminotransferase (ALT), HCV RNA levels and HCV genotype distribution.

PNPLA3 rs738409 genotype distribution

The genotype frequencies of PNPLA3 (rs738409) did not deviate from Hardy-Weinberg Equilibrium in all the studied participants. The genotype distributions and allele frequencies of the SNP are presented in Table 2. The frequencies of CC, CG and GG genotypes in HCV mono-infected patients were 53.2%, 40.3% and 6.5%, respectively, while the corresponding genotypes were 50.8%, 39.2%, and 10.0% in the HCV/HIV co-infected patients. Moreover, the corresponding genotype frequencies were 45.5%, 44.0% and 10.5% in the healthy controls. Compared with the healthy control group, HCV mono-infected patients had lower distribution of G allele [odds ratio (OR) =0.73, 95% confidence interval (CI) =0.55-0.99, P=0.043]. However, the genotype and allele frequencies were similar between controls and patients with HCV/HIV co-infection. In addition, the genotype distributions and allele frequencies were not different between the HCV mono-infected and HCV/HIV co-infected groups.

COX-2 rs689465 genotype distribution

The genotype distributions and allele frequencies of COX-2 (rs689465) are shown in Table 2. In the HCV mono-infected group, the frequencies of AA, AG and GG genotypes were 78.2%, 19.5% and 2.3%, respectively. The corresponding genotypes was 76.0%, 21.0% and 3.0% in the HCV/HIV co-infection group, while their distribution in the healthy control group were 57.5%, 32.5% and 10.0%, respectively. Our results showed that the frequencies of AG and GG genotypes were less common in the HCV mono-infection group compare with healthy controls (OR=0.44, 95%CI=0.28-0.71; P<0.001 and OR=0.17, 95%CI=0.06-0.46; P<0.001, respectively). Similar results were found among HCV/HIV co-infected patients compared with the healthy controls regarding the distribution of AG and GG genotypes (OR=0.49, 95%CI=0.31-0.77; P=0.002 and OR= 0.23, 95%CI=0.09-0.58; P=0.002, respectively). However, the genotype distributions and allele frequencies were not different between the HCV mono-infected and HCV/HIV co-infected groups (Table 2).

DHCR7 rs12785878 genotype distribution

The frequency of TT, TG and GG genotypes of DHCR7 (rs12785878) in each group are shown in Table 2. In the HCV mono-infected group, their frequencies were 56.8%, 38.2% and 5.0%, respectively, while their distribution in the HCV/HIV co-infected group were 67.0%, 39.5% and 3.5%, respectively. The corresponding genotypes in the healthy control group were 46.0%, 42.0% and 12.0%, respectively. The GG genotype was less frequently distributed in the HCV mono-infected group than in the healthy control group (OR=0.34, 95% CI=0.16-0.72, P=0.005). Likewise, GG genotype was less common in the HCV/HIV co-infected group than the healthy control group (OR=0.24, 95%CI=0.10-0.57, P=0.0001). However, the genotype distributions and allele frequencies were not different between the HCV mono-infected and HCV/HIV co-infected groups (Table 2).

Independent and additive effects of the SNPs associated with advanced liver fibrosis

To identify factors associated advanced liver fibrosis (F3 and F4, LSM≥9.5 kPa), baseline characteristics including patient's age, gender, ALT, HIV co-infection, HCV RNA viral load, HCV genotype, SNPs rs738409, rs689465 and rs12785878 were evaluated by logistic regression analyses. The data showed that age, HIV co-infection, SNPs rs738409 and rs689465 were associated with advanced liver fibrosis in univariate and multivariate analyses (Table 3).

The combined effect of the risk genotypes, including

Table 1. Baseline Characteristics of the Participants in This Study

	HCV mono-infection (n=220)	HCV/HIV co-infection (n= 200)	Healthy control (n=200)	P
Age (years)	43.1±10.4	42.6±7.2	47.5±5.2	<0.001
Gender				
Male (%)	155 (70.5%)	179 (89.5%)	111 (55.5%)	<0.001
Female (%)	65 (29.5%)	21 (10.5%)	89 (44.5%)	
ALT (IU/L)	71.5± 57.4	75.4± 61.3	ND	0.872
HCV-RNA (log_{10} IU/ml)	6.2± 2.1	6.3± 1.9	ND	0.554
HCV genotypes			ND	0.372
1	75 (34.1%)	76 (38.0%)		
3	105 (47.7%)	80 (40.0%)		
6	40 (18.2%)	30 (15.0%)		
Unknown	0(0%)	14 (7.0%)		
Fibrosis stage			ND	<0.001
F0-F1/F2	150 (68.2%)	97 (48.5%)		
F3/F4	70 (31.8%)	103 (51.5%)		
Liver stiffness (kPa)	10.2±8.6	13.3±10.5	ND	0.001

HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; ND, No data; Fibrosis stage cutoff; F0-F1 (<7.1 kPa), F2 (7.1-9.4 kPa), F3 (9.5-14.0) and F4 (>14.0 kPa).

Table 2. Genotype and Allele Frequencies of the Studied SNPs in Patients with HCV Infection and Controls

SNPs	HCV (n=220)	HCV-HIV (n=200)	Control (n=200)	HCV vs. control		HCV-HIV vs. control		HCV-HIV vs. HCV	
				OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
PNPLA3									
Allelic model									
Major (C)	325	282	270	1		1		1	
Minor (G)	115	118	130	0.73 (0.55-0.99)	0.043*	0.87 (0.64-1.17)	0.359	1.18 (0.87-1.60)	0.277
Additive model									
CC	120	102	91	1		1		1	
CG	85	78	88	0.73 (0.49-1.10)	0.131	0.79 (0.52-1.20)	0.268	1.08 (0.72-1.62)	0.711
GG	15	20	21	0.54 (0.26-1.11)	0.094	0.85 (0.43-1.67)	0.636	1.57 (0.76-3.22)	0.22
Dominant model									
CC	120	102	91	1		1		1	
CG+GG	100	98	109	0.70 (0.47-1.02)	0.064	0.80 (0.54-1.19)	0.271	1.15 (0.79-1.69)	0.467
Recessive model									
CC+CG	205	180	179	1		1		1	
GG	15	20	21	0.62 (0.31-1.25)	0.181	0.95 (0.50-1.81)	0.869	1.52 (0.755-3.05)	0.241
COX-2									
Allelic model									
Major (A)	387	346	295	1		1		1	
Minor (G)	53	54	105	0.38 (0.27-0.55)	<0.001*	0.44 (0.31-0.63)	<0.001*	1.14 (0.76-1.71)	0.528
Additive model									
AA	172	152	115	1		1		1	
AG	43	42	65	0.44 (0.28-0.70)	<0.001*	0.49 (0.31-0.77)	0.002*	1.11 (0.69-1.78)	0.682
GG	5	6	20	0.17 (0.06-0.46)	<0.001*	0.23 (0.09-0.58)	0.002*	1.36 (0.41-4.54)	0.619
Dominant model									
AA	172	152	115	1		1		1	
AG+GG	48	48	85	0.38 (0.25-0.58)	<0.001*	0.43 (0.28-0.66)	<0.001*	1.13 (0.72-1.78)	0.595
Recessive model									
AA+AG	215	194	180	1		1		1	
GG	5	6	20	0.21 (0.08-0.57)	0.002*	0.28 (0.11-0.71)	0.007*	1.33 (0.40-4.43)	0.642
DHCR7									
Allelic model									
Major (T)	334	307	268	1		1		1	
Minor (G)	106	93	132	0.64 (0.48-0.87)	0.004*	0.62 (0.45-0.84)	0.002*	0.96 (0.69-1.31)	0.774
Additive model									
TT	125	114	92	1		1		1	
TG	84	79	84	0.74 (0.49-1.10)	0.138	0.76 (0.50-1.15)	0.19	1.03 (0.69-1.54)	0.88
GG	11	7	24	0.34 (0.16-0.72)	0.005*	0.24 (0.10-0.57)	0.001*	0.70 (0.26-1.86)	0.472
Dominant model									
TT	125	114	92	1		1		1	
TG+GG	95	86	108	0.65 (0.44-0.95)	0.027*	0.64 (0.43-0.95)	0.028*	0.99 (0.67-1.46)	0.97
Recessive model									
TT+TG	209	193	176	1		1		1	
GG	11	7	24	0.39 (0.18-0.81)	0.012*	0.27 (0.11-0.63)	0.003*	0.69 (0.26-1.81)	0.451

OR, odd ratio; CI, confidence interval

rs738409 (CG+GG) and rs689465 AG+GG) on the presence of advanced liver fibrosis were further investigated. Among patients with advanced liver fibrosis, there were 66 (39.1%) patients who did not carry any risk genotype, while there were 67 (37.6%), 31 (53.4%) and 9 (60.0%) patients who had 1, 2 and 3 risk genotypes, respectively (P=0.044, Chi-square test for trend analysis). These data showed that the percentage of patients with advanced liver fibrosis increased significantly along with the accumulated numbers of the risk genotypes.

Table 3. Univariate and Multivariate Regression Analyses of Factors Associated with Advanced Liver Fibrosis (LSM ≥ 9.5 kPa)

Factors	Category	Univariate analysis		Multivariate analysis	
		Odd ratio (95%CI)	P	Odd ratio (95%CI)	P
Baseline					
Age (years)	< 40 vs. ≥ 40	4.08 (2.65-6.27)	<0.001*	4.44 (2.82-6.98)	<0.001*
Sex	Male vs. Female	1.59 (0.96-2.62)	0.07		
ALT (U/L)	< 75 vs. ≥ 75	0.98 (0.42-2.30)	0.965		
HIV co-infection	yes vs. no	2.28 (1.53-3.38)	<0.001*	2.39 (1.56-3.67)	<0.001*
Log10 HCV RNA (IU/mL)	< 6.0 vs. ≥ 6.0	1.10 (0.57-2.15)	0.81		
HCV genotypes	1 vs. 3 and 6	1.01 (0.82-1.24)	0.92		
SNPs					
PNPLA3 (rs738409)	GG vs. non-GG	1.42 (1.01-2.02)	0.049*	1.48 (1.01-2.16)	0.045*
COX-2 (rs689465)	GG vs. non-GG	2.59 (1.20-5.61)	0.016*	2.63 (1.19-5.80)	0.017*
DHCR7 (rs12785878)	GG vs. non-GG	0.84 (0.51-1.38)	0.491		

ALT, alanine aminotransferase; SNPs, Single nucleotide polymorphisms; CI, confidence interval

Discussion

Natural history and clinical outcome of HCV infection display remarkably inter-individual differences. Previous data have shown that more than 70% of acute HCV infection progress to chronic infection, in which approximately 20% of cases will develop towards cirrhosis and finally HCC (Lemoine and Thursz, 2014). Increasing data have indicated that the clinical course and disease progression of HCV infection is related to interaction of various factors including viral, host and environmental factors (Matsuura and Tanaka, 2017). Regarding host genetic variations, previous genome-wide association studies (GWAS) have shown that SNPs near the interferon lambda-3 (IFNL3) and interferon lambda-4 (IFNL4) genes are predictors of spontaneous HCV clearance and response to pegylated interferon-based therapy (Tanaka et al., 2009; Prokunina-Olsson et al., 2013). Recent GWAS have also identified additional genetic variations associated with the severity of liver fibrosis and HCC development (Matsuura et al., 2017).

In this report, multivariate analysis showed that PNPLA3 GG genotype was an independent risk factor associated with advanced liver fibrosis assessed by LSM. Indeed, LSM with transient elastography appears to be a reliable non-invasive tool to detect significant fibrosis or cirrhosis in patients with chronic HCV infection (Houot et al., 2016). Our results are in agreement with previous reports demonstrating that PNPLA3 polymorphism influenced the progression of liver fibrosis in HCV mono-infected and HCV/HIV co-infected patients (Trepo et al., 2011; Ali et al., 2016; Jimenez-Sousa et al., 2016; Nunez-Torres et al., 2016). For instance, an analysis of data from participants in a large cohort of HCV mono-infection showed that PNPLA3 genotype was significantly associated with the presence of cirrhosis after adjusting for other factors (Ali et al., 2016). In addition, a recent study reported that the presence of PNPLA3 (rs738409) G allele increased the odds of having advanced liver fibrosis in HCV/HIV co-infected patients (Jimenez-Sousa et al., 2016). In contrast, an association between the PNPLA3 polymorphism and the degree of liver fibrosis was not confirmed in other reports of HCV mono- and co-infection (Nakamura et al., 2013; Sagnelli et al., 2016).

COX-2 represents an inducible enzyme that converts arachidonic acid to prostaglandins, which are potent mediators of inflammation involved in several cellular processes including proliferation, carcinogenesis and metastasis (Simmons et al., 2004). Our data showed that COX-2 (rs689465) GG genotype was identified as a predictor of advanced liver fibrosis by multivariate analysis. This result was in accordance with a Japanese study demonstrating that rs689465 polymorphism was linked to liver disease progression in patients with HCV mono-infection (Miyashita et al., 2012). Moreover, a recent meta-analysis have suggested that COX-2 (rs689465) variant might be a factor associated with HCC risk in Asian populations (Bu and Zhao, 2013). Interestingly, our data also show for the first that the combined testing of PNPLA3 (rs738409) and COX-2 (rs689465) could exhibit an additive effect towards the presence of advanced liver fibrosis. Taken together, these results indicate that PNPLA3 and COX-2 variants might play a significant role in the progression of liver fibrosis in HCV mono-infected and HCV/HIV co-infected patients.

The mechanism underlying this association remains unclear as PNPLA3 does not seem to have a direct impact on the natural history of chronic HCV infection. In fact, rs738409 GG allele might not directly affect the PNPLA3 mRNA levels but it could result in an inhibition of PNPLA3 function and influence towards susceptibility to intrahepatic fat accumulation. As a result, it could be explained that PNPLA3 variant promotes liver fat accumulation, which in turn leads to progressive steatosis and, ultimately, the development of liver fibrosis and cirrhosis (Trepo et al., 2011). Regarding the role of COX-2, previous data suggested that patients with risk allele of rs689465 had higher expression levels of COX-2 mRNA in the liver and lymphoid cells (Miyashita et al., 2012). It was also showed that COX-2 over-expression in the hepatocytes was associated with advanced liver

fibrosis in patients with chronic HCV infection (Nunez et al., 2004). Thus, it is speculated that COX-2 GG genotype might promote higher levels of COX-2 expression, resulting greater liver inflammation and progressive liver fibrosis.

It has been recently showed that vitamin D, a potent immune-modulator, is associated with the pathogenesis of various disorders, including chronic HCV infection (Grunhage et al., 2012). In fact, hypovitaminosis D is frequently found among patients with chronic HCV infection. In our previous report, vitamin D insufficiency and deficiency were found in more than 60% of patients with HCV mono- and co-infection (Avihingsanon et al., 2014). In addition, common SNPs in vitamin D-related genes, such as DHCR7, CYP27B1, vitamin D receptor (VDR) and vitamin D binding protein (DBP), are linked to clinical manifestation and treatment response in patients with chronic HCV infection (Grunhage et al., 2012). For instance, a recent Italian report found an association between severity of liver fibrosis and DHCR7 variant in patients with HCV genotype 1 infection (Petta et al., 2013). In contrast, our cohort did not show that DHCR7 genotypes influenced progressive liver fibrosis in patients with chronic HCV infection. This discrepancy between studies might be related to the heterogeneity of reports in terms of studied population, genetic background, sample size, HCV genotypes, and differences in methods of liver fibrosis assessment. In light of this inconsistency, the roles of vitamin D-related SNPs in the clinical outcome of chronic HCV infection need to be elucidated in further studies.

In conclusion, PNPLA3 (rs738409) and COX-2 (rs689465) polymorphisms were associated with advanced liver fibrosis, suggesting that these variants might play an important role in liver fibrogenesis in patients with HCV mono- and co-infection. Given a higher chance for developing advanced fibrosis/cirrhosis and subsequent complications, patients harboring these risk genotypes may warrant closely monitored the clinical progression and being prioritized for antiviral therapy. Further cohorts with larger sample sizes should be performed to confirm these observations.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgements

This work was funded by the Grant for Chula Research Scholar (CU-GRS-60-06-30-03) and the 90th Anniversary of Chulalongkorn University Fund, Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University. The study was also supported by Center of Excellence in Hepatitis and Liver Cancer, Faculty of Medicine, Chulalongkorn University.

References

Ali M, Yopp A, Gopal P, et al (2016). A variant in PNPLA3 associated with fibrosis progression but not hepatocellular carcinoma in patients with hepatitis C virus infection. *Clin Gastroenterol Hepatol*, **14**, 295-300.

Avihingsanon A, Jitmitraparp S, Tangkijvanich P, et al (2014). Advanced liver fibrosis by transient elastography, fibrosis 4, and alanine aminotransferase/platelet ratio index among Asian hepatitis C with and without human immunodeficiency virus infection: role of vitamin D levels. *J Gastroenterol Hepatol*, **29**, 1706-14.

Bu X, Zhao C (2013). The association between cyclooxygenase-2 1195 G/A polymorphism and hepatocellular carcinoma: evidence from a meta-analysis. *Tumour Biol*, **34**, 1479-84.

Castera L, Pinzani M, Bosch J (2012). Non invasive evaluation of portal hypertension using transient elastography. *J Hepatol*, **56**, 696-703.

Falleti E, Fabris C, Cmet S, et al (2011). PNPLA3 rs738409C/G polymorphism in cirrhosis: relationship with the aetiology of liver disease and hepatocellular carcinoma occurrence. *Liver Int*, **31**, 1137-43.

Grunhage F, Hochrath K, Krawczyk M, et al (2012). Common genetic variation in vitamin D metabolism is associated with liver stiffness. *Hepatology*, **56**, 1883-91.

Hajarizadeh B, Grebely J, Dore GJ (2013). Epidemiology and natural history of HCV infection. *Nat Rev Gastroenterol Hepatol*, **10**, 553-62.

Houot M, Ngo Y, Munteanu M, et al (2016). Systematic review with meta-analysis: direct comparisons of biomarkers for the diagnosis of fibrosis in chronic hepatitis C and B. *Aliment Pharmacol Ther*, **43**, 16-29.

Intaraprasong P, Siramolpiwat S, Vilaichone RK (2016). Advances in management of hepatocellular carcinoma. *Asian Pac J Cancer Prev*, **17**, 3697-703.

Jimenez-Sousa MA, Berenguer J, Garcia-Alvarez M, et al (2016). Impact of patatin-like phospholipase domain-containing 3 gene polymorphism (rs738409) on severity of liver disease in HIV/hepatitis C virus-coinfected patients. *AIDS*, **30**, 465-70.

Lemoine M, Thursz M (2014). Hepatitis C, a global issue: access to care and new therapeutic and preventive approaches in resource-constrained areas. *Semin Liver Dis*, **34**, 89-97.

Matsuura K, Sawai H, Ikeo K, et al (2017). Genome-wide association study identifies TLL1 variant associated with development of hepatocellular carcinoma after eradication of hepatitis C virus infection. *Gastroenterology*, **152**, 1383-94.

Matsuura K, Tanaka Y (2017). Host genetic variations associated with disease progression in chronic hepatitis C virus infection. *Hepatol Res*, **48**, 127-33.

Miyashita M, Ito T, Sakaki M, et al (2012). Genetic polymorphism in cyclooxygenase-2 promoter affects hepatic inflammation and fibrosis in patients with chronic hepatitis C. *J Viral Hepat*, **19**, 608-14.

Nakamura M, Kanda T, Nakamoto S, et al (2013). No correlation between PNPLA3 rs738409 genotype and fatty liver and hepatic cirrhosis in Japanese patients with HCV. *PLoS One*, **8**, e81312.

Nunez-Torres R, Macias J, Mancebo M, et al (2016). The PNPLA3 genetic variant rs738409 influences the progression to cirrhosis in HIV/Hepatitis C virus coinfected patients. *PLoS One*, **11**, e0168265.

Nunez O, Fernandez-Martinez A, Majano PL, et al (2004). Increased intrahepatic cyclooxygenase 2, matrix metalloproteinase 2, and matrix metalloproteinase 9 expression is associated with progressive liver disease in chronic hepatitis C virus infection: role of viral core and NS5A proteins. *Gut*, **53**, 1665-72.

Petta S, Grimaudo S, Marco VD, et al (2013). Association of vitamin D serum levels and its common genetic determinants, with severity of liver fibrosis in genotype 1 chronic hepatitis C patients. *J Viral Hepat*, **20**, 486-93.

Prokunina-Olsson L, Muchmore B, Tang W, et al (2013). A

variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nat Genet*, **45**, 164-71.

Sagnelli C, Merli M, Uberti-Foppa C, et al (2016). Impact of PNPLA3 variants on liver histology of 168 patients with HIV infection and chronic hepatitis C. *Clin Microbiol Infect*, **22**, 372-8.

Simmons DL, Botting RM, Hla T (2004). Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. *Pharmacological Rev*, **56**, 387-437.

Tanaka Y, Nishida N, Sugiyama M, et al (2009). Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet*, **41**, 1105-9.

Trepo E, Pradat P, Potthoff A, et al (2011). Impact of patatin-like phospholipase-3 (rs738409 C>G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C. *Hepatology*, **54**, 60-9.

Trepo E, Romeo S, Zucman-Rossi J, et al (2016). PNPLA3 gene in liver diseases. *J Hepatol*, **65**, 399-412.

Zhang Y, Wang X, Liu Y, et al (2012). The GC, CYP2R1 and DHCR7 genes are associated with vitamin D levels in northeastern Han Chinese children. *Swiss Med Wkly*, **142**, w13636.