The Association between Genetic Variations in *PNPLA3* Gene (rs738409) and Severity of Liver Fibrosis in Thai Patients with Chronic Hepatitis C

ความสัมพันธ์ระหว่างความหลากหลายทางพันธุกรรมของยีน *PNPLA3* กับความรุนแรงของพังผืดในตับในผู้ป่วยไทยที่ติดเชื้อไวรัสตับอักเสบซีแบบเรื้อรัง

Rattanaporn Kiatbumrung (รัตนพร เกียรติบำรุง)* Dr.Jarika Makkoch (ดร.จาริกา มากคช)**
Dr.Sunchai Payungporn (ดร.สัญชัย พยุงภร)*** Pisit Tangkijvanich (พิสิฐ ตั้งกิจวานิชย์)****

ABSTRACT

Patatin-Like phospholipase domain containing protein 3 (*PNPLA3*) rs738409 (C>G) polymorphism has been associated with disease progression in non-alcoholic fatty liver disease. However, the role of the polymorphism in chronic hepatitis C virus (HCV) infection and co-infection with human immunodeficiency virus (HIV) remains unclear. Two hundred HCV mono-infection patients, 130 patients with HCV-HIV co-infection, 72 HCV spontaneous clearance patients and 200 healthy people controls were enrolled in this study. The polymorphism on the region of rs738409 was detected by allelic discrimination using real-time PCR with *Taq*Man probes. Liver fibrosis was assessed by transient elastography. The results revealed the frequency of CC, CG and GG genotypes of rs738409 in the HCV group were 111 (55.5%), 76 (38%) and 13 (6.5%) respectively, while those in co-infection group were 62 (47.7%), 52 (40.0%) and 16 (12.3%) respectively, and those in HCV spontaneous clearance group were 26 (36.1%), 33 (45.8%) and 13 (18.1%), respectively. In the control group, the corresponding genotypes were 91 (45.5%), 88 (44%) and 21 (10.5%). The frequency of non-CC genotype (CG and GG) was significantly higher in the control group than in the HCV group (odds ratio 1.49, 95% confidence interval: 1.00-2.21, *P*=0.0459). There was no difference in mean stiffness score between with CC and non-CC genotypes in HCV group compared to co-infection group. In addition, the frequency of advanced fibrosis (defined as a stiffness score of > 9.5 kPa) that was comparable between patients with CC and non-CC genotypes in HCV patients was not difference (32.4% vs. 31.5%, *P*=0.884), which was similar to co-infection patients (56.5% vs. 55.9%, *P*=0.948). In conclusion, rs738409 polymorphism was not associated with the severity of liver fibrosis in Thai patients with HCV mono-infection and HCV-HIV co-infection.

บทคัดย่อ

ความหลากหลายทางพันธุกรรมของยีน Patatin-Like phospholipase domain containing protein 3 หรือ *PNPLA3* ตำแหน่ง rs738409 มีความสัมพันธ์กับการดำเนินโรคตับคั่งไขมัน อย่างไรก็ตามการศึกษาความหลากหลายทางพันธุกรรมในผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีแบบเรื้อรังและผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีร่วมกับเอชไอวียังมีไม่มาก ในการศึกษานี้จึงใช้ผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีแบบเรื้อรัง 200 คน ผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีร่วมกับเอชไอวี 130 คน ผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีและสามารถหายได้เอง 72 คน และกลุ่มควบคุมที่มีสุขภาพดี 200 คน

* *Student, Master of Science Program in Biochemisty, Faculty of Medicine, Chulalongkorn University*

** *Post-Doctoral Researcher, Department of Biochemisty, Faculty of Medicine, Chulalongkorn University*

*** *Assistant Professor, Department of Biochemisty, Faculty of Medicine, Chulalongkorn University*

**** *Professor, Department of Biochemisty, Faculty of Medicine, Chulalongkorn University*

ตรวจสอบความหลากหลายทางพันธุกรรมตำแหน่ง rs738409 ด้วยวิธี allelic discrimination ใช้เครื่องเรียลทามพีซีอาร์โดย *Taq*Man probes และตรวจการเกิดพังผืดที่ตับโดยใช้เครื่อง transient elastography จากการศึกษาพบความถี่จีโนไทป์ CC GG และ CG ในกลุ่มผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีแบบเรื้อรังเป็น 111 (55.5%) 76 (38%) และ13 (6.5%) กลุ่มผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีร่วมกับเอชไอวีเป็น 62 (47.7%) 52 (40.0%) และ 16 (12.3%) กลุ่มผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีและสามารถหายได้เองเป็น 26 (36.1%) 33 (45.8%) และ 13 (18.1%) และในกลุ่มควบคุมพบ (45.5%) 88 (44%) และ 21 (10.5%) ตามลำดับ โดยพบความถี่จีโนไทป์ในกลุ่ม non-CC (CG และ GG) ในกลุ่มควบคุมมากกว่าผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีแบบเรื้อรังอย่างมีนัยสำคัญทางสถิติโดยมีอัตราเสี่ยงสัมพันธ์เท่ากับ 1.49 ที่ระดับความเชื่อมั่น 95 เท่ากับ 1.00-2.21 P=0.0459 แต่ไม่พบความแตกต่างของการเกิดพังผืดที่ตับระหว่างจีโนไทป์ CC และ non-CC ในกลุ่มผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีแบบเรื้อรังและผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีร่วมกับเอชไอวี เมื่อพิจารณาผู้ป่วยที่มีพังผืดที่ตับในระดับรุนแรง (> 9.5 กิโลปาสคาล) เปรียบเทียบความถี่ของจีโนไทป์ CC ต่อ non-CC ในกลุ่มผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีแบบเรื้อรังที่พบ 32.4% ต่อ 31.5% P=0.884 ในกลุ่มผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีร่วมกับเอชไอวีพบ 56.5% ต่อ 55.9% P=0.948 โดยสรุปความหลากหลายทางพันธุกรรมตำแหน่ง rs738409 ไม่สัมพันธ์กับระดับความรุนแรงของการเกิดพังผืดในตับในผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีแบบเรื้อรังและผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีร่วมกับเอชไอวี

Key Words: Hepatitis C virus (HCV), liver fibrosis, PNPLA3, SNP

คำสำคัญ: ไวรัสตับอักเสบซี พังผืดที่ตับ ยีน PNPLA3 ความหลากหลายทางพันธุกรรม

Introduction

Liver fibrosis and cirrhosis normally process in liver as the consequence of a sustained wound healing which responded to chronic liver injury from various causes including hepatitis virus, autoimmune, drug induced, cholestasis and metabolic diseases (Koike, 2007). This process could be initiated by liver injury, which would be resulted in the releasing of Reactive Oxygen species (ROS), acting as fibrogenic cytokine to activate Kupffer cell and Hepatic Stellate cell (HSC). After that, hepatic parenchyma would be substituted by its extracellular matrix proteins, mostly consisted of collagen. The substitution process can cause the forming of fibrous scar and develop into liver fibrosis, cirrhosis which increases intrahepatic resistance to blood flow, resulting in hepatic insufficiency and portal hypertension. Finally, the liver fibrosis can account for the hepatic transformation into hepatocellular carcinoma (HCC), which is the end stage liver disease and death (Bataller & Brenner, 2005). Liver biopsy was a gold standard for liver histology, disease activity and liver fibrosis measurement, but many factor can account for the limitations of liver biopsy, such as the error from sample, interobserver variability and risk for bleeding or pain in patients (Friedman, 2003). In recent years, there are alternative ways to evaluate stage of liver fibrosis instead of liver biopsy. These drawbacks have led to a search for alternative ways to evaluate and stage the degree of liver fibrosis. Transient elastography (TE, Fibroscan®) has been used for testing the severity of liver fibrosis with a very good sensitivity and specificity for diagnose stage of liver fibrosis. Importantly, this method is an effective non-invasive patient diagnosis (Afdhal, 2013; Sandrin et al., 2003).

Hepatitis C virus (HCV) infection is a major cause for chronic liver disease and can develop into cirrhosis and HCC. There are 170 million people worldwide have infected by HCV. Several factors have been associated with increasing risk of cirrhosis, including male sex, time of HCV infection, and alcohol consumption (Asselah, Rubbia-Brandt, Marcellin, & Negro, 2006). The 20 to 30% of HCV infection will develop into cirrhosis and approximately 1 to 4% of which can develop into HCC annually, which can be the cause of death. Overall, half of patients who developed cirrhosis will die from their liver disease (Benvegnu, Gios, Boccato, & Alberti, 2004). In previous study, the results found a fast progression of liver disease in patients with HCV co-infected with HIV who have record of blood transfusion, blood contamination and drug abuse. A meta-analysis study showed that the co-infection of HCV and HIV can bring about 2.92 folds of relative risk in cirrhosis compared to HCV mono-infected patients due to impetus effects of HIV infection on the natural history of HCV infection, such as increased HCV replication, decreased rate of HCV clearance, forced to liver fibrosis, increased frequency of liver failure and death (Macías et al., 2009; Martín-Carbonero et al., 2004).

Patatin-Like Phospholipase Domain-Containing Protein 3 (*PNPLA3*) was identified as a membrane-associated, adipose-enriched protein which is induced during adipogenesis mechanism. *PNPLA3* encodes 481 amino acids sequence and functions as lipase activity to break down the triglycerides. *PNPLA3* expresses in several types of human tissue but highly expresses in liver hepatic stellate cell (Basantani et al., 2011). Recently, genetic host factors have been hypothesized to influence liver fibrosis development. One of considerable factors is a single nucleotide polymorphism (SNP) on rs738409 (C>G), which can alter the amino acid Isoleucine into Methionine at codon 148. The 3D structures of 148M model examined the effect of substitution on the side chain of the hydrophobic substrate-binding groove in the active site, resulted in the decrease of lipase activity, followed by the increased hepatic triglyceride content (He et al., 2010).

This SNP has been reported its significant association with liver damage, liver fibrosis and cirrhosis in HCV patients. Variation of *PNPLA3* gene contributed to ethnic and inter-individual differences in hepatic fat content (Valenti et al., 2011). The *PNPLA3* 148M mutant allele can affect to HCC by increasing of fat content in liver tissue, leading to proinflammation and decrease of anti-inflammation and usually ends up in cirrhosis (Valenti, Dongiovanni, Ginanni Corradini, Burza, & Romeo, 2013). On the other hand, The study in Japan in 2013 reported that there was no correlation between *PNPLA3* rs738409 genotype and hepatic steatosis or liver fibrosis in Japanese patients infected with HCV (Nakamura et al., 2013).

Objective of the study

The aim of this study was to determine the association between single nucleotide polymorphisms in *PNPLA3* gene and severity of liver fibrosis in Thai patients with HCV mono-infection and HCV-HIV co-infection.

Materials and methods

Patients

Two hundred patients with HCV mono-infection, 72 patients with HCV spontaneous clearance in

King Chulalongkorn Memorial Hospital and 130 HCV co-infection patients from the HIV Netherlands Australia Thailand Research Collaboration involved this study. All patients registered in the liver clinic between October 2011 and October 2014. The patients were classified as; patients with HCV mono-infection (anti-HCV positive and HCV RNA positive); patients with HIV-HCV co-infection (anti-HCV positive and anti-HIV positive) and patients with HCV spontaneous clearance (anti-HCV positive and HCV RNA negative). All blood samples were performed for genotyping. Patients with hepatitis B surface antigen positive and presence of other cancer were excluded from this study. We also excluded patients without information on HCV genotype, and HCV viral load. Control samples were subjected from The Thai Red Cross Society of 200 Thai populations who enrolled and pass for the blood donor criteria. Written informed consents were obtained from all participants. The research protocol was approved by the ethics committees of the Research Affairs Chulalongkorn University (IRB 412/57).

Liver stiffness measurement

All patients with HCV mono-infection and HCV-HIV co-infection were evaluated the severity of liver fibrosis by measurement of liver stiffness with transient elastography (TE, Fibroscan®) according to the liver stiffness that from the National Institutes of Health. Histological stage of liver fibrosis can be divided in 5 stage, F0 = no fibrosis, F1 = mild, F2 = moderate, F3 = severe and F4 = cirrhosis. Liver stiffness was classified in F0-F1<7.1, F2<9.5, F3<12.5 and F4>14.5 kPa, respectively.

Genotyping

Human genomic DNA of each patient was extracted from Peripheral Blood Mononuclear Cells (PBMCs) by phenol chloroform isoamyl alcohol extraction. The *PNPLA3* (rs738409) C>G (cat.C_____7241_10) SNP, which encoded I148M, was genotyped by *Taq*Man SNP genotyping allelic discrimination method (Applied Biosystems, Foster City, CA). PCR was performed with Perfect Taq Master Mix (5 PRIME, Darmstadt, Germany) in 10 µl in total volume as recommended by the manufacturer. PCR conditions were described as follows: 95°C for 10 seconds, followed by 40 cycles at 92°C for 15 seconds and 60°C for 60 seconds. Allelic discrimination plot was evaluated by fluorescent signals (VIC and FAM) using the ABI Step One Plus real-time PCR system (Applied Biosystems).

Statistical Analysis

Data were presented as the mean ± standard deviation of each categorical variable (patient characteristics and stage of liver stiffness) while the fibrosis stage and genotype distribution data were presented in frequencies and percentages. Frequencies (%) of each genotype (CC, GG and CG) in patient were compared to those in healthy control using online GraphPad Software (http://www.graphpad.com). We evaluated the association between the rs738409 genotype and liver fibrosis incidence by using a binary variable and the univariate odds ratios (OR) to compare between CC genotype and non-CC genotype using MedCal Software (http://www.medcalc.org). The relationship between rs738409 polymorphisms and stage of liver fibrosis findings to analyze the association with genotyping of *PNPLA3* and severity of liver fibrosis in HCV mono-infection and HCV-HIV co-infection patients were also examined. The results were considered as significant when $P < 0.05$ (two-tailed) by chi-square test using SPSS software version 16.0.

Results

Patient Characteristics and Genotype frequencies of PNPLA3

Two hundred HCV mono-infection patients, one hundred and thirty with HCV-HIV co-infected patients and seventy-two HCV spontaneous clearance patients were enrolled in this study. The characteristics of the patients are showed in Table 1. Frequencies of PNPLA3 rs738409 genotype (CC, GG and CG) in HCV group were 111 (55.5%), 76 (38%) and 13 (6.5%) respectively, those in co-infection group were 62 (47.7%), 52 (40.0%) and 16 (12.3%), those in HCV spontaneous clearance group were 26 (36.1%), 33 (45.8%) and 13 (18.1%), respectively. In the control group, the corresponding genotypes were 91 (45.5%), 88 (44%) and 21 (10.5%).

Table 1 Patient Characteristics

	HCV	HCV/HIV	HCV clearance	Healthy control
N	200	130	72	200
Age	42.9±10.3	43.0±7.1	42.6±10.2	47.4±5.1
Sex (male)	141 (70.5%)	120 (92.3%)	38 (52.7%)	111 (55.5%)
Fibrosis stage				
F0 = no fibrosis	47.5%	23.1%	-	-
F1 = mild	18.0%	13.1%		
F2 = moderate	2.5%	7.7%		
F3 = severe	10.0%	24.6%		
F4 = cirrhosis	22.0%	31.5%		

Data are presented as mean ± standard deviation.

Table 2 Distribution of *PNPLA3* rs738409 (C>G) genotype

Genotype	HCV n=200	HCV/HIV n=130	HCV clearance n=72	Control n=200	HCV vs Control		HCV/HIV vs Control		HCV clearance vs Control	
					OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
GG	13 (6.5%)	16 (12.3%)	13 (18.1%)	21 (10.5%)	1	-	1	-	1	-
CG	76 (38.0%)	52 (40.0%)	33 (45.8%)	88 (44.0%)	1.39 (0.65-2.97)	0.3885	0.77 (0.37-1.61)	0.4981	0.61 (0.27-1.34)	0.2189
CC	111 (55.5%)	62 (47.7%)	26 (36.1%)	91 (45.5%)	1.97 (0.93-4.15)	0.0744	0.89 (0.43-1.84)	0.7628	0.46 (0.20-1.04)	0.0638
Non-CC	89 (44.5%)	68 (52.3%)	46 (63.89%)	109 (54.4%)	1	-	1	-	1	-
CC	111 (55.5%)	62 (47.7%)	26 (36.1%)	91 (45.5%)	1.49 (1.00-2.21)	0.0459*	1.09 (0.70-1.70)	0.6964	0.67 (0.38-1.18)	0.1688

95% confidential interval (CI); OR, odds ratio

Table 3 Frequencies of rs738409 genotype and stage of liver fibrosis in HCV mono-infection and HCV-HIV co-infection patients.

Patients	Genotype	Liver stiffness (mean ± SD)	F0-F2	F3-F4	chi-square	*P*-value
HCV (n=200)	CC (n=111)	11.84±10.78	75 (67.6%)	36 (32.4%)	0.021	0.884
	Non-CC (n=89)	10.72±9.66	61 (68.5%)	28 (31.5%)		
HCV/HIV (n=130)	CC (n=62)	15.04±11.90	27 (43.5%)	35 (56.5%)	0.004	0.948
	Non-CC (n=68)	13.93±9.99	30 (44.1%)	38 (55.9%)		

Data are presented as mean ± standard deviation.

The frequency of non-CC genotype (CG and GG) was significantly higher in the control group than those in the HCV group (odds ratio 1.49, 95% confidence interval: 1.00-2.21, *P*=0.0459) but no difference between HIV-HCV co-infection patients and HCV spontaneous clearance patients (Table 2). The SNP genotype distribution was in Hardy-Weinberg equilibrium (*P* value was non-significant, data not shown).

Association with severity of liver fibrosis

The clinical findings of liver stiffness were showed in Table 1. The frequencies of liver stiffness in each patients group were demonstrated in the graph. In HCV mono-infection group, the proportion of liver stiffness (mean±SD) tended to be mild to moderate fibrosis stage (F0-F2). In addition, in HCV-HIV co-infection patients, the mean of liver stiffness was high in patients with severe or advance fibrosis stage (F3-F4) (Fig.1). The Frequencies of advance fibrosis (F3-F4, defined as a stiffness score of > 9.5 kPa) score compared between CC and non-CC in HCV patients were 32.4% and 31.5% (*P*=0.884) and in HCV-HIV co-infection patients were 56.5% and 55.9%, *P*=0.948, respectively (Table 3). There was no difference in mean stiffness score between with CC and non-CC genotypes in both patient groups. The results showed that no significant association between rs738409 genotype and severity of liver fibrosis in patients with HCV mono-infection and patients with HCV-HIV co-infection.

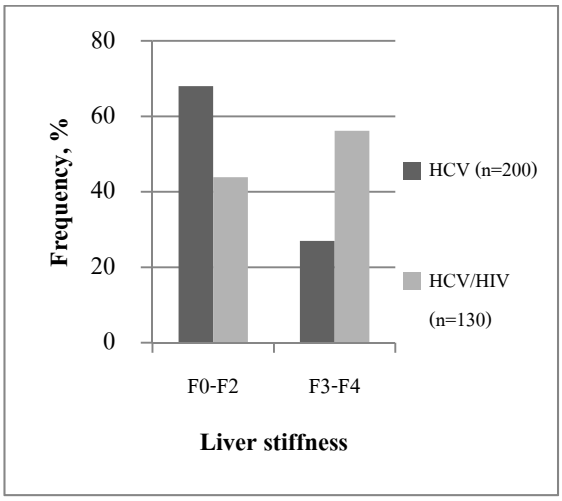

Figure 1 The frequency (%) of fibrosis stage in HCV mono-infection and HCV-HIV co-infection patients.

Discussion and conclusions

In this reserch project, we determined the correlation between the single nucleotide polymorphism in *PNPLA3* rs738409 (C>G) and severity of liver fibrosis in Thai patients with HCV mono-infection

and HCV-HIV co-infection patients. Recent study, liver fibrosis is frequently observed in HCV patients and it is influenced by several factors, such as alcohol consumption, BMI, obesity, diabetes, drug abuse and HCV infection (Friedman, 2003; Macías et al., 2009). Risk and natural history of fibrosis associated with HCV infection have been evaluated as a result of several large clinical studies. Considerably, single nucleotide polymorphism (SNP) might be factor that correlated with fibrosis progression in HCV. (Falleti et al., 2013)

Previously, rs738409 has been reported to have the association with progression of non-alcoholic fatty liver disease (NAFLD) but the role in this SNP remained unclear. The association between SNP and liver disease were also reported in many studies. The study in Germany reported that *PNPLA3* (rs738409 C>G) polymorphism favored the steatosis and fibrosis progression in HCV patients while mutant G allele homozygote carriers became the higher risk for steatosis (odds ratio [OR] 2.55, 95% confidence interval [CI] 1.08-6.03, P = 0.034), fibrosis (OR 3.13, 95% CI 1.50-6.51, P = 0.002), and fibrosis progression (OR 2.64, 95% CI 1.22-5.67, P = 0.013) (Trépo et al., 2011). The study in Italy found that the rs738409 GG genotype was associated with fibrosis stage and cirrhosis (OR 1.47, 95% CI 1.2-1.9; P = 0.002), treatment response (n = 470; OR 0.63, 95% CI 0.4-0.8; P = 0.006), and HCC occurrence (n = 325; OR 2.16, 95% CI 1.3-3.6; P = 0.002). The SNP "GG" genotype was risky genotype for steatosis development in HCV patients and was independently associated with cirrhosis and other steatosis-related clinical outcomes, such as lack of response to antiviral treatment and possibly HCC (Valenti et al., 2011).

In contrast, our results showed that the frequencies of non-CC genotype in *PNPLA3* (rs738409) was significantly higher in healthy control than those in HCV mono-infection group, but there were no difference compared to those in HCV-HIV co-infection group. In addition, the frequencies of stage liver fibrosis in HCV group were high in mild to moderate fibrosis stage (F0-F2). Inversely, the mean of liver stiffness in co-infection patients was high in severe or advance fibrosis stage (F3-F4). We also found no association between genotype variation on rs738409 and severity of liver fibrosis in HCV mono-infection and HCV-HIV co-infection patients. These findings were in accordance with a recent previous study. The study in Japan reported there was no association between *PNPLA3* rs738409 genotype and hepatic steatosis or liver fibrosis in Japanese patients who were infected with HCV based on Ultrasound findings (Nakamura et al., 2013).

The different results between the previous studies and ours might be described by the different ethnicity or different distribution of *PNPLA3* (rs738409) amoung groups of population which might have various association with severity of liver fibrosis. Many factors should be considered as the possible explanations of the discrepancy correlated with fibrosis progression such as; 1) viral load or genotype, these factors greatly impact to disease progression and response to antiviral therapy. 2) Human promoter polymorphisms (e.g. TGFb1 and angiotensin) might correlate with fibrosis risk. 3) Host immune response might be essential for fibrosis progression. (Friedman, 2003)

The different detection methods could affect different results such as liver biopsy vs fibroscan. In previous descript, liver biopsy might possible to

distort the results by sampling error, unsuitable sample size and requisite of the histologist to analyses tissue while fibroscan gained better sensitivity and specificity than liver biopsy (Sandrin et al., 2003). Genotyping methods, especially allelic discrimination with *Taq*Man genotyping assay and restriction fragment length polymorphism (RFLP) were basic method for SNP detection. There are many advantages and disadvantages in both methods which should be considered. The advantages of *Taq*Man genotyping assay were it was highly specific and sensitive but this method required specific equipment such as real-time PCR instrument and still be cost-consuming. RFLP is one of good options with lower cost but some of its limitations are lower specificity and sensitivity. Moreover, this method can present the error from DNA band in gel electrophoresis and from incubation period of enzyme (Chuang et al., 2008). However, some factors should be considered as limitations of this study, such as body mass index (BMI), alcohol consumption, smoking habits and diabetes, due to the difficulties in HIV samples recruitment.

In conclusion, the present study demonstrated that rs738409 polymorphism was not associated with the severity of liver fibrosis in Thai patients with HCV mono-infection and co-infection. The results in this study were based on fibroscan findings, which presented a greater accuracy and considered as a non-invasive method for the diagnosis of patients with high specificity and sensitivity in detection method. Importantly, this study was the first report about relationship between rs738409 and severity of liver fibrosis in Thai patients with HCV-HIV co-infection.

Acknowledgment

We express our gratitude to The Ratchadaphiseksomphot Endowment Fund of Chulalongkorn University (RES560530155) (90th Year Chulalongkorn Scholarship) for funding support. We also thank Research Unit of Hepatitis and Liver Cancer, department of biochemistry, faculty of medicine Chulalongkorn University for valuable help in data collection and funding and Lab 724 for technical assistance.

References

Afdhal NH. FibroScan in the Diagnosis of Hepatitis C Virus Infection. Gastroenterology and Hepatology. 2013; 9(8): 533-535.

Asselah T, Rubbia-Brandt L, Marcellin P, & Negro F. Steatosis in chronic hepatitis C: Why dose it really matter? Gut. 2006; 55(1): 123-130.

Basantani MK, Sitnick MT, Cai L, Brenner DS, Gardner NP, Li JZ, et al. Pnpla3/Adiponutrin deficiency in mice does not contribute to fatty liver disease or metabolic syndrome. Journal of Lipid Research. 2011; 52(2): 318-329.

Bataller R, & Brenner DA. Liver fibrosis. The Journal of Clinical Investigation. 2005; 115(2): 209-218.

Benvegnu L, Gios M, Boccato S, & Alberti A. Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complications. Gut. 2004; 53(5): 744-749.

Chuang LY, Yang CH, Tsui KH, Cheng YH, Chang PL, Wen CH, et al. Restriction enzyme mining for SNPs in genomes. Anticancer Res. 2008; 28(4A): 2001-2007.

Falleti E, Cmet S, Fabris C, Fattovich G, Cussigh A, Bitetto D, et al. Genetic polymorphisms of vitamin D pathway predict antiviral treatment outcome in slow responder naive patients with chronic hepatitis C. PLoS One. 2013; 8(11): e80764.

Friedman SL. Liver fibrosis -- from bench to bedside. J Hepatol. 2003; 38 Suppl 1: S38-53.

He S, McPhaul C, Li JZ, Garuti R, Kinch L, Grishin NV, et al. A Sequence Variation (I148M) in PNPLA3 Associated with Nonalcoholic Fatty Liver Disease Disrupts Triglyceride Hydrolysis. Journal of Biological Chemistry. 2010; 285(9): 6706-6715.

Koike K. Hepatitis C virus contributes to hepatocarcinogenesis by modulating metabolic and intracellular signaling pathways. J Gastroenterol Hepatol. 2007; 22 Suppl 1: S108-111.

Macías J, Berenguer J, Japón MA, Girón JA, Rivero A, López-Cortés LF, et al. Fast fibrosis progression between repeated liver biopsies in patients coinfected with human immunodeficiency virus/hepatitis C virus. Hepatology. 2009; 50(4): 1056-1063.

Martín-Carbonero L, Benhamou Y, Puoti M, Berenguer J, Mallolas J, Quereda C, et al. Incidence and Predictors of Severe Liver Fibrosis in Human Immunodeficiency Virus—Infected Patients with Chronic Hepatitis C: A European Collaborative Study. Clinical Infectious Diseases. 2004; 38(1): 128-133.

Nakamura M, Kanda T, Nakamoto S, Miyamura T, Jiang X, Wu S, et al. No Correlation between PNPLA3 rs738409 Genotype and Fatty Liver and Hepatic Cirrhosis in Japanese Patients with HCV. PLoS ONE. 2013; 8(12): 1-7.

Sandrin L, Fourquet B, Hasquenoph J-M, Yon S, Fournier C, Mal F, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound in medicine & biology. 2003; 29(12): 1705-1713.

Trépo E, Pradat P, Potthoff A, Momozawa Y, Quertinmont E, Gustot T, et al. Impact of patatin-like phospholipase-3 (rs738409 C>G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C. Hepatology. 2011; 54(1): 60-69.

Valenti L, Dongiovanni P, Ginanni Corradini S, Burza MA, & Romeo S. PNPLA3 I148M variant and hepatocellular carcinoma: A common genetic variant for a rare disease. Digestive and Liver Disease. 2013; 45(8): 619-624.

Valenti L, Rumi M, Galmozzi E, Aghemo A, Del Menico B, De Nicola S, et al. Patatin-Like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. Hepatology. 2011; 53(3): 791-799.