Research Article

JOURNAL OF HEPATOLOGY

Association of diabetes and *PNPLA3* genetic variants with disease severity of patients with chronic hepatitis C virus infection

Chung-Feng Huang[1,2,3,4,†], Chia-Yen Dai[2,4,7,†], Ming-Lun Yeh[2,5], Ching-I Huang[2], Chi-Ming Tai[5,6], Meng-Hsuan Hsieh[7], Po-Cheng Liang[2], Yi-Hung Lin[2], Ming-Yen Hsieh[2,9], Hua-Ling Yang[2], Jee-Fu Huang[2,4,8], Zu-Yau Lin[2,4], Shinn-Cherng Chen[2,4], Ming-Lung Yu[2,4,10,*,†], Wan-Long Chuang[2,4,*,†]

[1]*Institute of Clinical Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan;* [2]*Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan;* [3]*Department of Occupational Medicine, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan;* [4]*Faculty of Internal Medicine, School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan;* [5]*Graduate Institute of Clinical Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan;* [6]*Department of Internal Medicine, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan;* [7]*Department of Preventive Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan;* [8]*Department of Internal Medicine, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan;* [9]*Department of Internal Medicine, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan;* [10]*Institute of Biomedical Sciences, National Sun Yat-Sen University, Kaohsiung, Taiwan*

Background & Aims: Genetic variants of patatin-like phospholipase domain-containing 3 (*PNPLA3*) and diabetes are associated with liver disease severity, in patients with chronic hepatitis C (CHC) infection. We aimed at exploring their interaction in determining hepatitis C virus (HCV)-related liver fibrosis.

Methods: The *PNPLA3* genetic polymorphism at rs738409 was verified in 1077 biopsy-proven CHC patients. Other clinical variables, including diabetes status, were analysed for factors associated with bridging fibrosis.

Results: Patients with advanced liver fibrosis had higher proportions of the GG genotype (14.5% *vs.* 10.4%, $p = 0.06$ in recessive model) and GG/GC genotype carriage (64.0% *vs.* 56.8%, $p = 0.03$ in dominant model). Stepwise logistic regression analysis revealed that factors predictive of advanced liver fibrosis included age (odds ratio [OR]: 1.02, 95% confidence intervals [CI]: 1.008–1.037, $p = 0.002$), diabetes (OR: 1.81, CI: 1.236–2.653, $p = 0.002$), α-fetoprotein (OR: 1.006, CI: 1.001–1.01, $p = 0.01$), platelet counts (OR: 1.009, CI: 1.006–1.012, $p < 0.001$), and *PNPLA3* rs738409 CG/GG genotype (OR: 1.34, CI: 1.006–1.785, $p = 0.046$). When patients were grouped according to their diabetes status, the *PNPLA3* genetic variants were associated with advanced liver fibrosis in diabetic patients only, but not in non-diabetic patients. The *PNPLA3* gene was the most important predictive factor of bridging fibrosis in diabetic patients, using the recessive model (OR: 4.53, CI: 1.356–15.106, $p = 0.014$) or the dominant model (OR: 2.20, CI: 1.026–4.734, $p = 0.04$). Compared to non-diabetic patients, patients with the diabetes/GG genotype were more likely to have advanced liver fibrosis (OR: 8.79, CI: 2.889–26.719, $p < 0.001$), followed by those with diabetes/non-GG genotype (OR: 1.55, CI: 1.048–2.286, $p = 0.03$).

Conclusions: The effect of *PNPLA3* genetic variants in HCV-related advanced liver fibrosis was enhanced in diabetic patients. The strong genetic–environmental interaction contributed to the high risk of advanced liver disease in CHC patients.

Keywords: HCV; CHC; Liver fibrosis; PNPLA3; SNP; DM.

Received 3 June 2014; received in revised form 6 October 2014; accepted 8 October 2014; available online 20 October 2014

* Corresponding authors. Address: Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Chung-Ho Memorial Hospital, No. 100, Tzyou 1st Road, Kaohsiung 807, Taiwan. Tel.: +886 7 312 1101x7475; fax: +886 7 312 3955.

E-mail address: fish6069@gmail.com (M.-L. Yu).

† These authors contributed equally to this work.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; APRI, aspartate aminotransferase-to-Platelet Ratio Index; AFP, α-fetoprotein; CHC, chronic hepatitis C; DM, diabetes mellitus; HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; *PNPLA3*, patatin-like phospholipase domain-containing 3; SNP, single-nucleotide polymorphism.

Introduction

More than 170 million people are chronically infected with hepatitis C virus (HCV) [1], which is one of the most important causes of liver cirrhosis and liver related mortality. The magnitude of disease progression in chronic HCV (CHC) infection varies significantly among individuals. Several factors have been recognized as being associated with the progression of HCV-related liver fibrosis and with clinical outcomes, including age and time since initial HCV infection [2], hepatitis B virus (HBV) or/and human

immunodeficient virus (HIV) co-infection, alcoholism, obesity, and hepatic steatosis [3,4], and non-response to antiviral therapy [5,6]. As liver fibrosis progression remains variable between individuals with similar environmental or virological risks, host genetic predispositions have been suggested as another critical determinant [7–13].

Patatin-like phospholipase domain-containing 3 (*PNPLA3*), known as adiponutrin, and the rs738409 C>G single-nucleotide polymorphism (SNP), which encodes for the I148M protein variant, have been well recognized as a genetic determinant of non-alcoholic fatty liver disease (NAFLD), in terms of inflammation and fibrosis [14–16]. Although the issue remains debated [17], genetic variants of *PNPLA3* have been associated with HCV-related liver disease severity [18–21].

The causal linkage of HCV and one of its extrahepatic manifestation, diabetes mellitus (DM), is complex [22]. From the perspective of the natural history of HCV, patients with insulin resistance might experience more rapid disease progression and adverse clinical outcomes [23]; DM is recognized as a risk factor for disease severity [24] and might be an independent predictor for liver cirrhosis [25]. It has been suggested that *PNPLA3* genetic variants do not determine insulin sensitivity [26] or metabolic syndrome component traits, including DM [27]. However, the issue of *PNPLA3* genetic susceptibility in patients with extreme characteristics has also been raised [28,29]. *PNPLA3*-related liver disease severity in CHC among Asians is rarely observed. The other genetic–environmental interaction, DM and *PNPLA3*, which influences HCV-related liver disease severity, has not been previously determined. Herein, we recruited a well-characterized appropriately sized HCV cohort, to determine the effect of the interaction between *PNPLA3* SNP and DM on liver fibrosis, in Asian patients.

Materials and methods

Patients

We retrospectively recruited 1077 consecutive CHC patients in a medical centre and two core regional hospitals in Southern Taiwan from 2001 to 2013. All patients planned to receive anti-viral therapy, and a liver biopsy was performed prior to treatment. Patients with HIV infection, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, Wilson disease, α_1-antitrypsin deficiency, a current or past history of alcohol abuse ($\geqslant$20 g daily), psychiatric condition, previous liver transplantation, or evidence of hepatocellular carcinoma were excluded. All medical histories and co-administered drugs were reviewed by the physicians and recorded by trained coordinators in the out-patient department. Anti-HCV antibodies were detected using a third-generation, commercially available enzyme-linked immunosorbent assay kit (AxSYM 3.0, Abbott Laboratories, Chicago, IL, USA). Serum HCV RNA was detected using real-time polymerase chain reaction (COBAS AMPLICOR Hepatitis C Virus Test, ver. 2.0; Roche, Branchburg, NJ, USA, detection limit: 50 IU/ml). The HCV genotypes were determined using the Okamoto method [30]. The biochemical parameters were measured on a multichannel auto-analyser (Hitachi Inc. Tokyo, Japan). The liver histology was graded and staged according to the scoring system described by Knodell and Scheuer [31], and steatosis was evaluated with an H&E stain. To overcome sampling variability in liver biopsies and avoid potential underestimation of the effect of *PNPLA3* on the progression of liver disease, we evaluated the association of the genetic variant in patients with bridging fibrosis (F3–4) rather than with cirrhosis alone [19]. The study was approved by the ethics committees at the participating hospitals and was performed according to the guidelines of the International Conference on Harmonization for Good Clinical Practice. All patients gave written informed consent before enrolment.

PNPLA3 rs738409 genotyping

PNPLA3 rs738409 genotyping was performed as described in our previous study [32]. Briefly, genomic DNA was extracted from EDTA whole-blood samples following standard procedures and was stored at −20 °C. Approximately 10 ng DNA was used for genotyping using the TaqMan PCR (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. Post-polymerase chain reaction allelic discrimination was performed by measuring the allele-specific fluorescence on an ABI Prism Sequence Detection System ABI 7900HT (Applied Biosystems). The primers were 5′-GCTTTCACAGGCCTTGGTATG-3′ (forward primer) and 5′-GGAGGGATAAGGCCACTGTAGAA-3′ (reverse primer). The probes were 5′-TTCCTGCTTCATCCC-3′ for Ile148 and 5′-TTCCTGCTTCATGCC-3′ for Met148. This assay is designed for the reverse strand, such that the G allele corresponds to the Met148 genotype when the gene is transcribed from the forward strand. All allele and genotype frequencies were consistent with Hardy–Weinberg equilibrium. The genotypic distribution was consistent with healthy Asian populations [32].

Statistical analyses

The frequency was compared between groups using the χ^2 test with the Yates correction or Fisher's exact test. Group means, presented as the mean values and standard deviations, were compared using the analysis of variance and Student's *t* test or the Mann–Whitney *U* test. The serum HCV RNA levels were expressed after logarithmic transformation of the original values. The influence of *PNPLA3* on liver fibrosis was calculated using recessive (genotype GG *vs.* CG + CC) and dominant (genotype CC *vs.* CG + GG) genetic models of inheritance. A stepwise logistic regression analysis was performed to evaluate the independent factors associated with advanced liver fibrosis by analysing the co-variants with p <0.05 in the univariate analysis. The statistical analyses were performed using the SPSS 12.0 statistical package (SPSS, Chicago, IL, USA). All statistical analyses were based on two-tailed hypothesis tests with a significance level of p <0.05.

Results

Patient profiles

The basic demographical, virological and clinical features of the 1077 patients are shown in Table 1. The mean age was 51.7 ± 11.5 years. Males accounted for 56.8% of the population. The mean HCV RNA levels were 5.40 ± 0.98 log IU/ml. The patient numbers in the groups from F0 to F4 were 119 (11.0%), 320 (29.7%), 299 (27.8%), 171 (15.9%), and 168 (15.6%), respectively. Patients with advanced fibrosis (F3–4) accounted for 31.5% of the population. The proportions of patients with *PNPLA3* rs738409 GG, GC, and CC genotypes were 11.7%, 47.4%, and 40.9%, respectively (Table 1).

Factors associated with bridging liver fibrosis

In the univariate analysis, factors associated with bridging liver fibrosis included older age, lower platelet count, higher α-fetoprotein (AFP) and alanine aminotransferase (AST) levels, and diabetes. Patients with advanced fibrosis had a higher proportion of *PNPLA3* risk G allele carriage. Compared to patients with mild liver disease, patients with advanced liver fibrosis had a higher proportion of GG genotype (14.5% *vs.* 10.4%, $p = 0.06$ in the recessive model) and GG/GC genotype carriage (64.0% *vs.* 56.8%, $p = 0.03$ in the dominant model) (Table 1). The stepwise logistic regression analysis showed that factors associated with advanced liver fibrosis in the dominant model included age (odds ratio/ [OR]: 1.02, 95% confidence intervals [CI]: 1.008–1.037, $p = 0.002$), diabetes (OR: 1.81, CI: 1.236–2.653, $p = 0.002$), AFP (OR: 1.006, CI: 1.001–1.01, $p = 0.01$), platelet count (OR: 1.009, CI: 1.006–1.012, p <0.001), and *PNPLA3* rs738409 CG/GG genotype (OR: 1.34, CI: 1.006–1.785, $p = 0.046$) (Table 2). Patients with GG genotype had significantly higher proportion of steatosis compared to their counterparts (64.5% *vs.* 41.6%, p <0.001). However, steatosis did not impact liver disease severity (Tables 1 and 2).