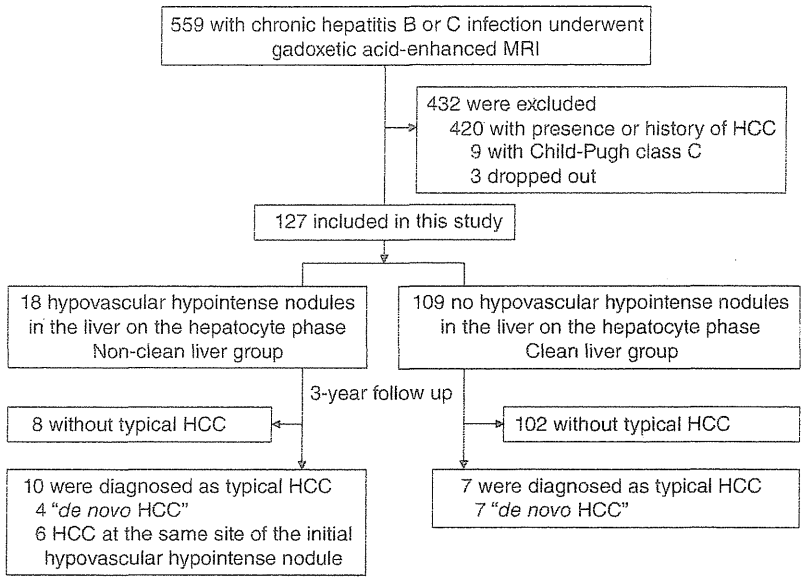


Figure 1 Patient inclusion criteria. "De novo HCC" is a typical hepatocelluiar carcinoma that developed at sites in which no nodules had been seen on the initial gadoxetic acid-enhanced magnetic resonance imaging (MRI).

modalities led to diagnosis of HCC, recognizing hypervascularization by more than one experienced radiologist and other imaging modalities was regarded as the time of diagnosis of HCC. When needle biopsy was performed to investigate nodules, the time of diagnosis of HCC was when the pathologists and physicians examined pathological tissue and diagnosed as HCC.

MRI

Magnetic resonance imaging was performed using a superconducting magnet that operated at 1.5 Tesla (Sigma EXCITE HD; GE Medical Systems, Milwaukee, WI, USA) and an 8-channel phased-array coil. First, we obtained fast spoiled gradient-echo T_1-weighted images (T1WI) with dual echo acquisition and respiratory-triggered fat-saturated fast spin-echo T_2-weighted images (T2WI). Dynamic fat-suppressed gradient-echo T1WI were obtained using a 3-D acquisition sequence before (precontrast) and 20–30 s, 60 s, 2 min, 5 min, 10 min and 20 min after the administration of gadoxetic acid (Primovist; Bayer Schering Pharma, Berlin, Germany). This contrast agent (0.025 mM/kg bodyweight) was administrated i.v. as a bolus at a rate of 1 mL/s through an i.v. cubital line (20–22 G) that was flushed with 20 mL saline from a power injector. The delay time for the arterial phase scan was adjusted according to a fluoroscopic triggering method.[20] All images were acquired in the transverse plane. Sagittal plane T1WI were also obtained during the hepatocyte phase at 20 min after the injection of the contrast agent.

Statistical analysis

All continuous values are expressed as median (range). Fisher's exact probability test was used for comparisons between categorical variable and the non-parametric Mann-Whitney *U*-test was used to compare differences between continuous variables. Baseline clinical characteristics, including blood test results, were evaluated within 1 month of the initial MRI. We investigated whether or not HCC development was associated with age, sex, fibrosis, etiology (HBV or HCV), platelet count, serum alanine aminotransferase (ALT), γ-glutamyltransferase (γ-GT), α-fetoprotein (AFP), and the presence or absence of hypovascular hypointense nodules.

Cumulative HCC development was estimated according to the Kaplan–Meier method and differences in the curves were tested using the log–rank test. Risk factors for HCC development were determined according to the Cox proportional hazard model. Subgroup analyses with a Cox proportional hazard model were applied to estimation of the hazard ratio (HR) of the non-clean liver group versus clean liver group in the dichotomized subgroups. All statistical analyses were performed using JMP software, version 10 (SAS Institute Japan, Tokyo, Japan). A two-sided *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Characteristics of the patients and nodules

A TOTAL OF 127 patients were enrolled, of whom 26 had chronic HBV infections and 101 had HCV infections, and 68 had virus-associated cirrhosis. No statistically significant differences in the initial clinical characteristics were found between the non-clean liver and clean liver groups (Table 1). Thirty-five hypovascular hypointense nodules were found in 18 patients in the non-clean liver group (1–5 nodules per patient) at baseline (data not shown). Twenty-four of these 35 nodules were detectable only on the hepatocyte phase MRI and were undetectable by US, CT and non-hepatocyte phase MRI. None of the 35 nodules showed high intensity on T2WI. The median nodule diameter was 8 mm (range, 4–13 mm; 33 nodules with ≤10 mm, two nodules with 12 mm and 13 mm).

HCC incidence according to initial MRI findings

Hepatocellular carcinoma was diagnosed in 17 patients, 10 in the non-clean liver group and seven in the clean liver group; 14 of these patients had HCV infection. Thirteen patients were diagnosed according to the AASLD imaging criteria.[19] Four patients were diagnosed pathologically by liver biopsies that were performed, based on enlargement of the nodules of more than 10 mm in diameter during the observation period.

The cumulative 1-, 2- and 3-year HCC incidence rates were 1.5%, 10.2% and 13.4%, respectively. As determined by the Kaplan–Meier method, these rates were 11.1% (95% confidence interval [CI], 0.0–25.6%), 38.8% (95% CI, 16.3–61.4%) and 55.5% (95% CI, 32.6–78.5%) in the non-clean liver group, and 0.0% (95% CI, 0.0–2.3%), 5.5% (95% CI, 0.0–9.8%) and 6.4% (95% CI, 1.8–11.0%) in the clean liver group; the former group showed significantly higher rates of development of typical HCC than the latter ($P < 0.001$) as shown in Figure 2. The median imaging intervals were 3 months (range, 3–6) in the non-clean liver group and 4 months (range, 2–12) in the clean liver group. The imaging interval of the non-clean liver group was shorter than the clean liver group (3 vs 4 months, $P = 0.015$). The median intervals between the initial MRI and HCC diagnosis was 16 months (range, 9–32) in the non-clean liver group and 21 months (range, 16–35) in the clean liver group.

In 11 of 17 patients with HCC development, HCC developed at sites in which no nodules had been seen on the initial gadoxetic acid-enhanced MRI, namely de novo HCC. These HCC were found in four of 18 patients in the non-clean liver group (3-year HCC incidence rates: 22.2%; 95% CI, 4.3–51.0%) and 7 in 109 patients in the clean liver group (3-year HCC incidence rates: 6.4%; 95% CI, 1.8–11.0%). The incidence rates of de novo HCC was significantly higher in the non-clean liver group than the clean liver group ($P = 0.003$, Fig. 3). In the remaining six patients, HCC developed at the same site of the initial nodules exclusively in 18 patients of a non-clean liver group by definition, and those HCC arose among the nodules of 8 mm or more in the initial MRI study.

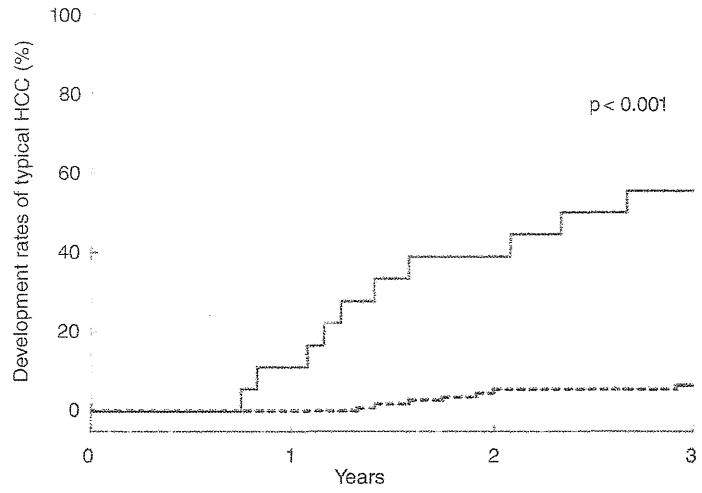
Risk factors for HCC development

Univariate analyses showed that the significant risk factors for HCC development included older age ($P = 0.039$), cirrhosis ($P = 0.009$), a low platelet count ($P = 0.003$), a high AFP concentration ($P = 0.006$) and a non-clean liver ($P < 0.001$). Multivariate analysis with these variables revealed that older age (hazard ratio [HR], 1.08; 95% CI, 1.01–1.16; $P = 0.024$), a low plate-

Table 1 Baseline patient characteristics

Characteristics	Total ($n = 127$)	Non-clean liver ($n = 18$)	Clean liver ($n = 109$)	P
Age, years	65 (30–88)	68 (46–82)	64 (30–88)	0.15
Male/female	68/59	10/8	58/51	1.00
Non-cirrhosis/cirrhosis	59/68	6/12	53/56	0.31
HBV/HCV	26/101	5/13	21/88	0.53
Platelet count ($\times 10^9$/L)	122 (30–410)	102 (46–187)	125 (30–410)	0.07
ALT (IU/L)	32 (7–206)	32 (14–95)	32 (7–206)	0.97
γ-GT (IU/L)	31 (9–305)	31 (13–258)	31 (9–305)	0.68
AFP (ng/mL)	4 (1–582)	8 (2–181)	4 (1–582)	0.19

Continuous data are shown as medians (range).
γ-GT, γ-glutamyltransferase; AFP, α-fetoprotein; ALT, alanine aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus.



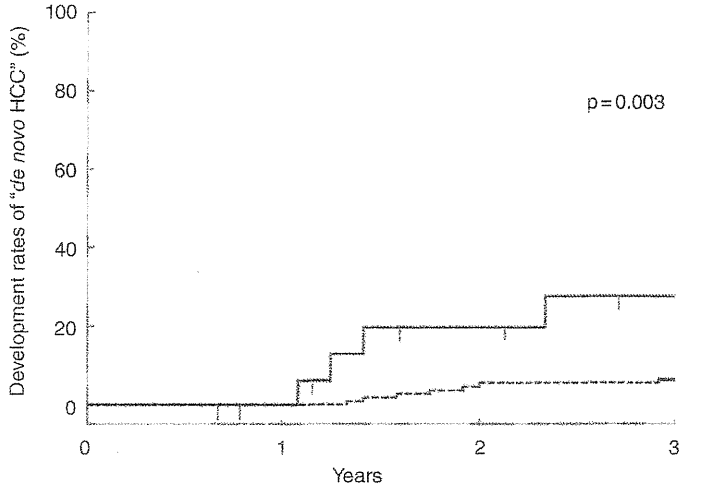
No. of patients at risk

Non-clean liver	18	16	11	8
Clean liver	109	109	103	102

Figure 2 Cumulative incidence rates of typical hepatocellular carcinoma (HCC) development in the non-clean and clean liver groups. ——, non-clean liver group ($n = 18$); - - -, clean liver group ($n = 109$).

let count (HR, 1.17; 95% CI, 1.03–1.35; $P = 0.017$) and a non-clean liver (HR, 9.41; 95% CI, 3.47–25.46; $P < 0.001$) were the only independent risk factors for HCC development (Table 2).

We further assessed the effect of a non-clean liver on the risk of HCC development in subgroups of these patients (Fig. 4). We found that belonging to the non-clean liver group was a significant risk factor in patients without HBV. Notably, this designation was particularly valuable for patients who are generally regarded as at low risk for HCC development: those without cirrhosis (HR, 37.23; 95% CI, 3.30–419.71; $P = 0.003$) and those with high platelet counts (HR, 33.42; 95% CI, 6.69–166.94; $P < 0.001$).



No. of patients at risk

Non-clean liver	18	18	15	14
Clean liver	109	109	103	102

Figure 3 Cumulative incidence rates of typical hepatocellular carcinoma (HCC) at sites in which no nodules had been seen on the initial gadoxetic acid-enhanced magnetic resonance imaging, namely, "de novo HCC". ——, non-clean liver group ($n = 18$); - - -, clean liver group ($n = 109$).

Table 2 Variables that predict HCC development: univariate and multivariate analyses

Variables	Univariate		Multivariate	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Male	0.56 (0.29–1.95)	0.755		
Age (per year)	1.06 (1.00–1.12)	0.039	1.08 (1.01–1.16)	0.024
Cirrhosis	14.37 (1.90–108.44)	0.009	3.54 (0.37–33.77)	0.231
HCV (vs HBV)	4.39 (0.58–33.17)	0.151		
Platelet count (per 10^{10}/L)	1.19 (1.06–1.33)	0.003	1.17 (1.03–1.35)	0.017
ALT (per IU/L)	1.00 (0.99–1.02)	0.423		
γ-GT (per IU/L)	1.00 (0.99–1.01)	0.688		
AFP >10 ng/mL	3.98 (1.47–10.77)	0.006	1.47 (0.49–4.33)	0.486
Non-clean liver	12.36 (4.68–32.61)	<0.001	9.41 (3.47–25.46)	<0.001

γ-GT, γ-glutamyltransferase; AFP, α-fetoprotein; ALT, alanine aminotransferase; CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

DISCUSSION

THIS STUDY REVEALED presence of hypovascular hypointense liver nodules (non-clean liver) on gadoxetic acid-enhanced MRI, is a significant risk factor for subsequent development of typical HCC not only at the same sites but also at the different sites from the initial nodules. The incidence of development of typical HCC in the non-clean liver patients was more than 50% during a 3-year follow-up period, indicating that these higher risk patients should be rigorously investigated for the early detection of HCC during follow up.

In the present study, six of the 18 patients in the non-clean liver group developed typical HCC at the same site of the initial nodules during the subsequent 3 years (11.1%/year). Most of the hypovascular hypointense nodules on gadoxetic acid-enhanced MRI are considered precursor lesions of typical HCC, such as early HCC or high-grade dysplastic nodules, on histological examination,[13–15] while it has been reported that most hypovascular nodules exhibiting high-intensity to isointensity signals in the hepatocyte phase are benign hepatic nodules.[14,15] Recent studies have suggested that a reduction of organic anion-transporting polypeptide 1B3 (OATP 8) transporter expression begins at the earliest stage of hepatocarcinogenesis,[21,22] before changes in vascularity such as decreased portal flow or increased arterial flow. The progression rate of the small

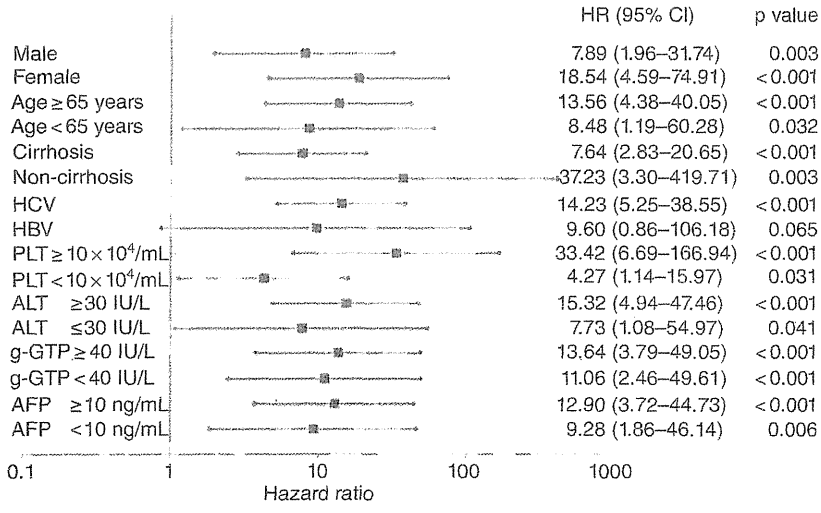


Figure 4 Stratified analyses of the non-clean liver as a risk factor for typical HCC development. AFP, α-fetoprotein; ALT, alanine aminotransferase; CI, confidence interval; g-GTP, γ-glutamyltransferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; PLT, platelets.

hypovascular hypointense nodules to typical HCC was reported as 10–17%/year,[9,10] which is comparable to the present study. Typical HCC arose exclusively among the nodules of 8 mm or more, as in previous studies in which the larger hypovascular hypointense nodules were found to be the risk factor for progression to typical HCC in the initial MRI study.[9,10]

Hyperintensity on T2WI[12] or diffusion-weighted images (DWI)[11] also was reported to be useful for prediction of typical HCC progress in hypovascular hypointense nodules. In our patients, none of the nodules in the non-clean liver group showed hyperintensity on T2WI, suggesting that the hepatocyte phase is more sensitive for detecting the early stage of hepatocarcinogenesis.[15] DWI were not evaluated in this study because this usually detects pathologically advanced HCC of larger size or with hypervascularity.[23] Thus, it is reasonable that the hepatocyte phase can effectively recognize the earliest stage of HCC development without T2WI or DWI.

In 11 of 17 patients, typical HCC developed at sites other than the initially detected hypovascular hypointense nodules. As shown in Figure 3, the incidence rates of such HCC in the non-clean liver group was significantly higher than in the clean liver group ($P = 0.003$), indicating that a non-clean liver itself is a risk factor for HCC development, apart from the detectable hypovascular hypointense nodules. In addition, in four patients with nodules even below 8 mm, two developed HCC at different sites from the initial nodules during follow up (data not shown). Taken together, a non-clean liver has the higher potential for hepatocarcinogenesis or for undetectable precursor lesions. The non-clean liver may reflect more advanced genetic or epigenetic changes in the background hepatocytes, however, the detailed biological mechanism is not clear in this study.

Non-clean liver was an independent risk factor for the development of typical HCC, apart from well-documented risk factors (Table 2), such as cirrhosis,[24] ALT,[25] γ-GT,[26] age and AFP.[27] A non-clean liver is a significant risk for HCC development also for those without cirrhosis or with high platelet counts (Fig. 4). This means patients at increased risk of HCC development can be discerned as having a non-clean liver even among low-risk subgroups.

Conversely, patients without such nodules (clean liver group) showed a significantly lower risk of developing typical HCC than those with non-clean livers (0.0% vs 11.1% at 1 year, 6.8% vs 55.5% at 3 years of follow up; $P < 0.001$), suggesting that gadoxetic acid-enhanced MRI could detect precursor lesions sensitively enough to rule out immediate (within 1 year) development of typical HCC. Although seven patients in the clean liver group developed typical HCC only after 1 year, these patients had other risk factors for HCC development, including lower platelet counts, implying more advanced liver cirrhosis or high AFP (data not shown). Such HCC may arise from precursor lesions that cannot be visualized by current imaging techniques.

This study is a retrospective study and has some limitations. We included patients with HBV and HCV together, because gadoxetic acid-enhanced MRI findings or HCC development do not differ between these two groups and HBV or HCV infection is not an independent risk factor for typical HCC development. However, the number of HBV patients was too small ($n = 26$) to statistically confirm the current result when limited to HBV patients only. Prospective studies with larger numbers of patients who have uniform liver disease etiologies and imaging intervals are needed to verify our findings in different settings. Although the imaging interval of the non-clean liver group was shorter than the clean liver group (3 vs 4 months: $P = 0.015$), the median intervals between the initial MRI and HCC diagnosis was 16 months in the non-clean liver group and 21 months in the clean liver group. They are short enough for cumulative detection of HCC development for 3 years and it is assumed that there was little influence on the conclusions.

In conclusion, patients with chronic viral liver disease are at high risk for developing typical HCC at any sites of the liver if they have hypovascular hypointense nodules on gadoxetic acid-enhanced MRI. These patients should be closely followed up for developing typical HCC not only at the same site but also at different sites from the initial nodule.

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Hepatology Research

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Original Article

Interferon lambda 4 polymorphism affects on outcome of telaprevir, pegylated interferon and ribavirin combination therapy for chronic hepatitis C

Yuko Nagaoki,[1] Michio Imamura,[1] Yoshiiku Kawakami,[1] Hiromi Kan,[1] Hatsue Fujino,[1] Takayuki Fukuhara,[1] Tomoki Kobayashi,[1] Atsushi Ono,[1] Takashi Nakahara,[1] Noriaki Naeshiro,[1] Ayako Urabe,[1] Satoe Yokoyama,[1] Daisuke Miyaki,[1] Eisuke Murakami,[1] Tomokazu Kawaoka,[1] Masataka Tsuge,[1] Akira Hiramatsu,[1] Hiroshi Aikata,[1] Shoichi Takahashi,[1] C. Nelson Hayes,[1] Hidenori Ochi[2] and Kazuaki Chayama;[1,2] Hiroshima Liver Study Group

[1]*Department of Gastroenterology and Metabolism, Applied Life Science, Institute of Biomedical & Health Science, Hiroshima University, and* [2]*Laboratory for Digestive Diseases, Center for Genomic Medicine, The Institute of Physical and Chemical Research (RIKEN), Hiroshima, Japan*

Aim: The predictive value of the recently identified interferon-λ (IFNL)4 polymorphism on the outcome of telaprevir (TVR), pegylated interferon (PEG IFN) plus ribavirin (RBV) combination therapy for chronic hepatitis C is unknown.

Methods: We assessed predictive factors for sustained virological response (SVR) for TVR, PEG IFN plus RBV combination therapy in 283 genotype 1 chronic hepatitis C patients. IFNL4 polymorphism ss469415590 was analyzed by Invader assay.

Results: SVR rates for patients with IFNL4 TT/TT genotype were significantly higher than for those with the IFNL4 TT/ΔG or ΔG/ΔG genotypes (93% and 59%, respectively, $P < 0.0001$). In a multivariate regression analysis, prior treatment history (treatment-naïve patients or patients who relapsed during prior treatment) (odds ratio [OR], 2.385; $P = 0.028$), rapid virological response (OR, 6.800; $P < 0.0001$) and ss469415590 TT/TT genotype (OR, 8.064; $P < 0.0001$) were identified as significant independent predictors for SVR. In patients with IFNL4 TT/ΔG or ΔG/ΔG genotypes, SVR rates for non-RVR patients were significantly lower than RVR patients (22% and 75%, respectively, $P < 0.0001$).

Conclusion: Analysis of IFNL4 polymorphism is a valuable predictor in patients receiving TVR triple therapy.

Key words: chronic hepatitis C, inteferon lambda 4, rapid virological response, sustained virological response, telaprevir

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is a common cause of chronic hepatitis and hepatocarcinogenesis worldwide.[1–3] To prevent the development of hepatocellular carcinoma and advanced liver disease, interferon (IFN)-based therapies are administrated to patients with chronic HCV infection. Telaprevir (TVR), a new direct-acting antiviral agent, has recently been approved in several countries. Patients with high viral load of genotype 1 are treated with a three-drug combination therapy of TVR, pegylated (PEG) IFN and ribavirin (RBV) for 24 weeks. Because TVR is a selective inhibitor of HCV non-structural 3/4A protease activity, marked improvement in sustained virological response (SVR) rates are expected.[4–8]

Correspondence: Professor Kazuaki Chayama, Department of Gastroenterology and Metabolism, Applied Life Sciences, Institute of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Email: chayama@hiroshima-u.ac.jp

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Recent genome-wide association studies have shown that common single nucleotide polymorphisms (SNP) near the interleukin-28B (*IL28B*) gene (rs8099917 and rs12979860) on chromosome 19 are strongly associated with spontaneous clearance of HCV infection and outcome of PEG IFN plus RBV combination therapy.[9–12] Furthermore, IL28B polymorphisms are also associated with triple therapy with TVR, PEG IFN and RBV.[13,14] More recently, Prokunina-Olsson *et al.* reported that a polymorphism (ss469415590) within a gene encoding the novel interferon-lambda 4 (IFNL4) protein is more strongly associated with HCV clearance and outcome after PEG IFN plus RBV combination treatment compared to rs12979860.[15,16] IFNL4 protein can be produced by individuals who carry the ΔG allele of the ss469415590 variant (IFNL4-ΔG) but not by individuals who are homozygous for the IFNL4-TT allele because of a frame-shift in exon 1 caused by an insertion variant.[15] The rs12979860 variant is located within intron 1 of IFNL4. Linkage disequilibrium is strong between the IFNL4-ΔG allele and the unfavorable rs12979860-T allele in individuals of European or Asian ancestry, whereas this linkage disequilibrium is moderate in individuals of African ancestry. Compared to rs12979860, ss469415590 is more strongly associated with HCV clearance in individuals of African ancestry, although it provides comparable information in Europeans and Asians.

In the present study, we assessed efficacy and predictive factors for SVR and the effect of the IFNL4 polymorphism on TVR, PEG IFN plus RBV triple therapy.

METHODS

Patients

A TOTAL OF 283 patients with chronic genotype 1 HCV infection who were treated with TVR, PEG IFN-α-2b plus RBV at Hiroshima University Hospital and hospitals belonging to the Hiroshima Liver Study Group were enrolled. All subjects gave written informed consent to participate in the study. Inclusion criteria for the therapy included remaining positive for genotype 1 HCV RNA for 6 months. Exclusion criteria included patients with cirrhosis. Patient characteristics are shown in Table 1. Patients were classified according to response to prior IFN therapy. Relapse was defined as undetectable HCV RNA by the end of the prior treatment, followed by a return to detectable HCV RNA levels after treatment had ended. Non-response was defined as continuously detectable HCV RNA throughout the prior treatment. In this study, only three out of 283 patients (1.1%) showed discrepant haplotypes (Table 2). All three patients showed genotypes with rs8099917 TT, ss469415590 TT/ΔG and rs1127354 CC. Two of the three patients achieved SVR, and the third showed no response.

Table 1 Patient characteristics

Total	283
Sex (male/female)	143/140
Age (years)	63 (25–79)
Bodyweight (kg)	60 (36.2–95.7)
Body mass index (kg/m^2)	23.4 (14.6–42.2)
HCV genotype (1a/1b)	1/282
Level of viremia (log IU/mL)	6.6 (1.2–7.8)
Aspartate aminotransferase (IU/L)	37 (5–200)
Alanine aminotransferase (IU/L)	36 (10–286)
γ-Glutamyltransferase (IU/L)	30 (9–669)
Serum creatinine (g/dL)	0.7 (0.42–1.80)
eGFR (%)	75.4 (30.6–145)
Leukocyte count (/mm^3)	4 900 (2 400–37 700)
Hemoglobin (g/dL)	13.8 (10–17.6)
Platelet count (×10^4/μL)	16.0 (5.2–40.0)
α-Fetoprotein (ng/L)	5.2 (1–35.5)
Total cholesterol (mg/dL)	172 (63–251)
Previous treatment response	
Naïve/relapser/NR	60/118/105
Initial TVR dose (2 250/1 500 mg/day)	162/118
Amino acid substitutions in HCV genotype 1b	
HCV Core70 wild (R)/mutant (Q or H)/ND	108/83/92
IL28B	
rs8099917 genotype (TT/TG+GG)	179/104
IFNL4 genotype	
ss469415590 genotype (TT/TT/TT/ΔG + ΔGΔG)	176/107
ITPA genotype	
rs1127354 genotype (CC/CA+AA)	279/4

Categorical data are represented as numbers of patients, and continuous data is represented as median and range.
HCV, hepatitis C virus; IFNL4, interferon-λ 4; ITPA, inosine triphosphate pyrophosphatase; ND, not determined; NR, non-responder; TVR, telaprevir.

HCV RNA levels

Hepatitis C virus RNA levels were measured using COBAS Taqman HCV test (Roche Diagnostics, Tokyo, Japan). The detection limit for the assay was 1.2 log IU/mL. HCV genotype was determined by sequence

Table 2 Characteristics of three patients with discrepant haplotypes

Case	Sex	Age, years	rs8099917 genotype	ss469415590 genotype	rs1127354 genotype	Previous treatment response	Treatment response
1	M	58	TT	TT/ΔG	CC	Relapser	RVR, SVR
2	F	58	TT	TT/ΔG	CC	NR	NR
3	M	63	TT	TT/ΔG	CC	Relapser	RVR, SVR

NR, non-responder; RVR, rapid virological response; SVR, sustained virological response.

determination in the 5′-non-structural region of the HCV genome followed by phylogenetic analysis. Amino acid substitutions at position 70 in the HCV core protein (core70) were determined using direct sequencing of polymerase chain reaction products after extraction and reverse transcription of HCV RNA as in Akuta *et al.*[17]

SNP genotyping

We genotyped each patient for three SNP: rs8099917 (IL28B); ss469415590 (IFNL4); and rs1127354, an inosine triphosphate pyrophosphate SNP reported to be associated with ribavirin-induced anemia.[18–21] Samples were genotyped using the Invader assay as described previously.[22,23]

Therapeutic protocol

All patients were administrated 750 mg of TVR (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan) either three times a day at 8-h intervals after meals (2250 mg/day) or twice per day (1500 mg/day). The TVR dose was determined by each physician according to age, sex, bodyweight and hemoglobin level. PEG IFN-α-2b (PEG-Intron; MSD, Tokyo, Japan) was injected s.c. at a median dose of 1.5 μg/kg (range, 1.32–1.71) once per week. RBV (Rebetol; MSD) 200–600 mg was administrated after breakfast and dinner. The RBV dose was adjusted by bodyweight (600 mg for <60 kg; 800 mg for 60–80 kg; and 1000 mg for >80 kg). The triple therapy with PEG IFN-α-2b, RBV and TVR was continued for 12 weeks and then switched to PEG IFN-α-2b and RBV dual therapy for an additional 12 weeks.

Efficacy of the treatment

Sustained virological response was defined as undetectable serum HCV RNA at 24 weeks after the end of treatment. Rapid virological response (RVR) was defined as undetectable HCV RNA at week 4 of treatment.

Statistical analysis

Continuous variables are presented using the median and range and were analyzed using the Mann–Whitney *U*-test. Categorical variables were compared using the χ^2-test or Fisher's exact test, as appropriate. Multivariate analysis was conducted with a Cox proportional hazard model using the stepwise selection of variables or two logistic analyses. All statistical analyses were performed using the SPSS software package (version 12.0 for Windows; SPSS, Chicago, IL, USA), with $P < 0.05$ denoting statistical significance.

RESULTS

Predictive factors associated with SVR

A TOTAL OF 283 patients with chronic genotype 1 HCV infection were treated with TVR, PEG IFN-α-2b plus RBV. Of these 283 patients, 226 (80%) achieved SVR. SVR was more likely to be achieved in 51 treatment-naïve patients (85%) and 111 patients who relapsed during prior treatment (94%) compared to 64 patients with non-response to prior treatment (61%) (Fig. 1). Significant univariate predictors for SVR included clinical factors (younger patients, aspartate aminotransferase, alanine aminotransferase, hemoglobin, platelet count, HCV core70 wild, rs8099917 genotype and ss469415590 genotype), previous treatment response and on-treatment factor (RVR) (Table 3). Multivariate logistic regression analysis identified naïve or prior relapser (odds ratio [OR], 2.385 for previous non-responders; $P = 0.028$), RVR (OR, 6.800 for non-RVR; $P < 0.0001$) and ss469415590 TT/TT genotype (OR, 8.064 for TT/ΔG + ΔG/ΔG genotype; $P < 0.0001$) as significant independent predictors for SVR. The median adherence of TVR, PEG IFN-α-2b and RBV was 67%, 88% and 52%, respectively. Adherence to any of the drugs was not identified as a significant independent predictor for SVR.

Effect of RVR on SVR

Rapid virological response was achieved in 75% of patients. The SVR rate was significantly higher for RVR patients than non-RVR patients (88% vs 56%,

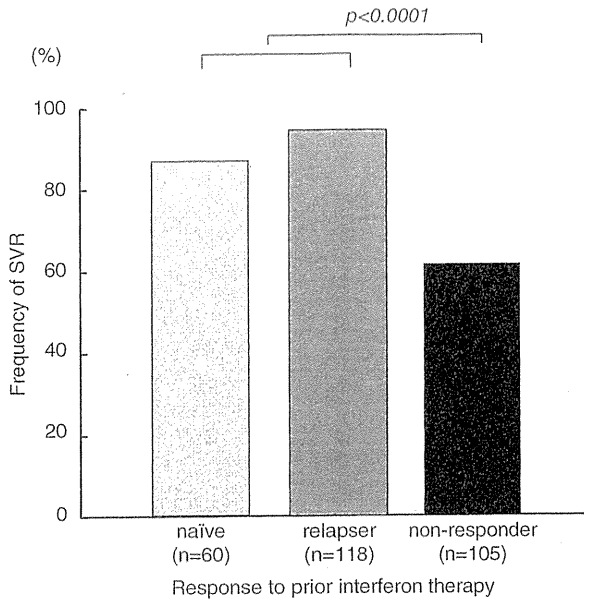

Figure 1 SVR for triple therapy grouped by response to prior interferon treatment. SVR, sustained virological response at 24 weeks after the end of therapy.

$P < 0.0001$) (Table 3). The RVR rate was significantly higher for treatment-naïve patients and prior relapsers than previous non-responders (82% vs 62%, $P < 0.0001$) (Table 3). In multivariate analysis, prior treatment history (OR, 2.807 for previous non-responders; $P < 0.0001$) was the only significant factor associated with RVR.

Effect of IFNL4 genotype and response to previous IFN treatment

We assessed the relationship between IFNL4 genotype and SVR. Patients with ss469415590 TT/TT genotype were significantly more likely to achieve SVR compared to those with TT/ΔG or ΔG/ΔG genotypes (93% vs 59%, $P < 0.0001$) (Table 3). Based on the response to previous IFN treatment, SVR rates for treatment-naïve patients and prior relapsers were significantly higher than for prior non-responders in both ss469415590 TT/TT (96% vs 81%, $P = 0.00285$) and TT/ΔG or ΔG/ΔG genotypes (74% vs 50%, $P < 0.0001$) (Fig. 2a).

Relationship of IFNL4 to SVR and RVR in non-responders to prior treatment

In patients with IFNL4 TT/TT genotype, achievement of RVR was not associated with SVR (96% vs 83%, $P = 0.00540$). In contrast, in patients with IFNL4 genotypes TT/ΔG or ΔG/ΔG, the SVR ratio was 75% in RVR patients but only 22% in non-RVR patients ($P < 0.0001$) (Fig. 2b). These results indicate that RVR was more likely to be associated with SVR in patients with IFNL4 TT/ΔG or ΔG/ΔG genotypes.

Effect of initial TVR dose on treatment response

In this study, 162 out of 283 (57%) patients were treated with an initial TVR dose of 2250 mg, and the remaining 43% of patients were treated with 1500 mg (Table 1). Initial TVR dose was not associated with SVR ratios either in IFNL4 TT/TT or TT/ΔG or ΔG/ΔG genotypes.

DISCUSSION

ALTHOUGH TRIPLE THERAPY improves the eradication rate of treated patients, adverse effects of the therapy may be severe.[4–8] Hence, it is important to predict the effect of the therapy as early as possible. This study was conducted to identify pretreatment factors associated with SVR for triple therapy. Overall, 226 of 283 patients (80%) achieved SVR, but treatment-naïve patients (85%) and prior treatment relapsers (94%) showed higher SVR rates than prior treatment non-responders (61%). This suggests that treatment-naïve patients as well as prior treatment relapsers may be good candidates for triple therapy without regard to other predictive factors, including IFNL4 genotype.

Single nucleotide polymorphism ss469415590 in the IFNL4 gene is strongly associated with HCV clearance through both the innate immune reaction and by PEG IFN plus ribavirin therapy.[15,16] This IFNL4 genotype is also important in predicting the probability of eradicating the virus by triple therapy. As shown in Table 3, patients with treatment-favorable IFNL4 genotype TT/TT showed a 93% eradication rate. Furthermore, the eradication rate was 81% even in patients with non-response to prior therapy (Fig. 2a). Accordingly, treatment with triple therapy is recommend for patients with this genotype. In contrast, among previously treated patients with unfavorable genotypes TT/ΔG or ΔG/ΔG, only treatment-naïve patients and prior relapsers had excellent SVR rates (74%, Fig. 2a). As IFNL4 SNP ss469415590 is in strong linkage disequilibrium with IL28B SNP rs8099917 and rs12979860,[15] only three out of 283 patients (1.1%) showed discrepant haplotypes in this study (Table 2). Among subgroups of patients with genotype concordance between rs8099917 and

Table 3 Univariate and multivariate logistic regression analysis of host and viral factors associated with SVR and RVR

	SVR ($n = 226$)	Non-SVR ($n = 57$)	Univariate analysis, *P*-value	RVR ($n = 211$)	Non-RVR ($n = 72$)	Univariate analysis, *P*-value
Sex (male/female)	112/114	31/26	0.555	106/105	37/35	0.692
Age (≤65/≥66)	154/72	30/27	0.031	137/74	47/25	1.000
Bodyweight (kg)	59.0 ± 11.0	59.5 ± 10.6	0.809	59.3 ± 10.6	60.8 ± 12.0	0.249
Aspartate aminotransferase (IU/L)	44 ± 28.3	58 ± 33.6	0.001	45 ± 29.2	52 ± 31.5	0.080
Alanine aminotransferase (IU/L)	46 ± 35.8	54 ± 37.1	0.029	46 ± 37.2	55 ± 41.5	0.521
eGFR(%)	77 ± 15.1	79 ± 20.6	0.473	77 ± 15.780	80 ± 20.3	0.207
Leukocyte count (/mm3)	5253 ± 1680	5286 ± 1456	0.116	5145 ± 2734	5263 ± 1754	0.008
Hemoglobin (g/dL)	14.4 ± 8.01	13.5 ± 1.37	0.047	14.4 ± 8.3	14.1 ± 1.4	0.431
Platelet count ($\times 10^4/\mu L$)	17.3 ± 5.62	12.8 ± 4.76	<0.0001	16.0 ± 5.5	15.8 ± 6.0	0.160
Platelet count ($\geq 15/< 15 \times 10^4/\mu L$)	136/89	19/38	<0.0001	120/90	35/37	0.220
Previous treatment response (naïve or relapse/NR)	162/64	16/41	<0.0001	146/65	32/40	<0.0001
Initial TVR dose (2 250 mg/1 500 mg)	127/98	35/20	0.364	119/90	43/28	0.697
TVR dose adherence (≥70%/<70%)	102/124	15/42	0.011	91/120	26/46	0.333
PEG IFN-α-2b dose adherence (≥80/<80%)	156/70	20/37	<0.0001	136/76	41/31	0.325
Ribavirin dose adherence (≥60/60%)	106/120	15/42	0.007	93/118	28/44	0.492
RVR/non-RVR	186/40	25/32	<0.0001	–	–	–
HCV Core70 wild (R)/mutant (Q or H)	97/55	11/28	<0.0001	81/61	27/22	0.868
rs8099917 genotype (TT/TG+GG)	165/61	14/43	<0.0001	138/73	41/31	0.206
ss469415590 genotype (TT/TΔG + ΔGΔG)	163/63	13/44	<0.0001	136/75	40/32	0.206
rs1127354 genotype (CC/CA+AA)	167/59	44/13	0.734	159/54	54/18	1.000

Continuous variables are expressed using the median and range.
eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; NR, non-responder; PEG IFN-α-2b, pegylated interferon-α-2b; RBV, ribavirin; RVR, rapid virological response; SVR, sustained virological response; TVR, telaprevir.

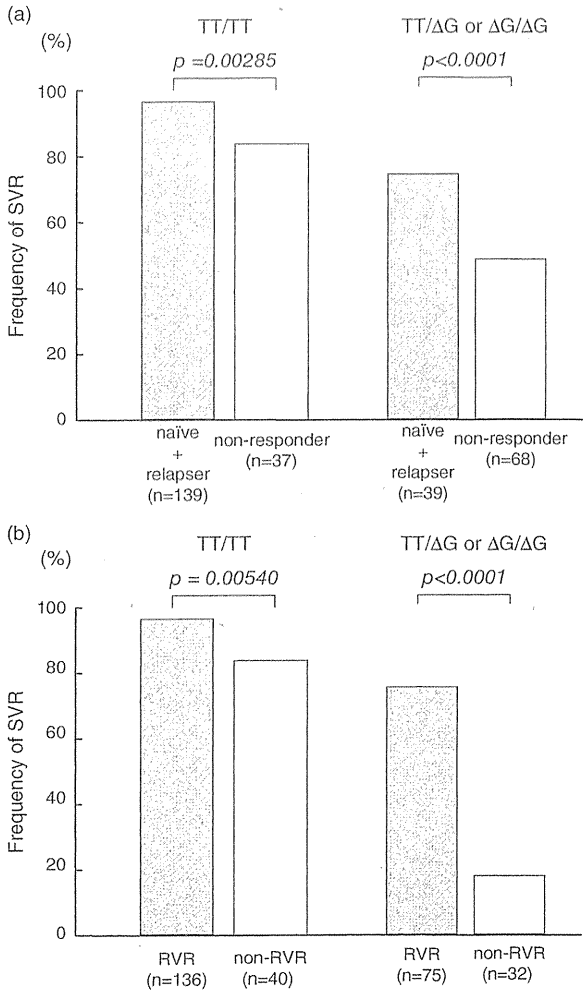

Figure 2 Relationship between interferon-λ (IFNL)4 polymorphism and sustained virological response (SVR). (a) The frequency of SVR for triple therapy grouped by ss469415590 genotype with respect to the response to prior interferon therapy and (b) with respect to rapid virological response (RVR).

ss469415590, the two SNP theoretically show identical association with treatment outcome irrespective of which SNP confers the best predictability in the population. In contrast, subgroup analysis of genotype discordant patients may be able to address the question of which SNP is the best predictor of treatment response even when the proportion of genotype concordant patients is very small. Although the small number of subjects with genotype discordance ($n = 3$) was not sufficient to provide statistically significant confirmation, our findings suggest that ss469415590 is a better predictor of treatment outcome than rs8099917 in patients with discordant SNP genotypes. It has been reported that the strength of linkage disequilibrium between the two SNP differs considerably among populations.[15] Considering that these two SNP are in strong linkage disequilibrium in the Japanese population, our results are not inconsistent with recent findings that ss469415590 is a functional variant modulating IFNL4 expression and is more strongly associated with viral clearance than previously reported IL28B SNP.[15] Bibert *et al.* has reported that TT/ΔG polymorphism (ss469415590) is a better predictor of spontaneous HCV clearance than rs12979860 and that induction of IL28B and IP-10 relies on TT/ΔG but not rs12979860 in Caucasian patients.[24] Thus, ss469415590 seems to consistently show the strongest association among studies, suggesting that the SNP is a true causal variant across populations.

More information is necessary for patients who are expected to have poor response to the therapy, such as patients with IFNL4 genotype TT/ΔG or ΔG/ΔG. For such patients, the rapid change in HCV RNA levels early in treatment is an important predictor of treatment success. More than 80% of patients who achieved RVR also achieved SVR. In contrast, patients with unfavorable IFNL4 genotypes who did not achieve RVR also showed poor response to the therapy; only seven out of 32 patients (22%) in this group achieved SVR (Fig. 2b). Accordingly, prolongation of the therapy may be considered when patients with IFNL4 genotypes associated with poor response fail to show RVR.

In the present study, initial TVR dose (2250 mg vs 1500 mg/day) was not associated with treatment response in patients with either the IFNL4 TT/TT genotype or the TT/ΔG or ΔG/ΔG genotypes. Although TVR improved the anti-HCV effect, severe adverse effects have also been reported.[4–6] The dose of TVR for use in triple therapy was determined based on a dose-finding study conducted in the USA and Europe,[25] which found that the 2250-mg regimen achieved the greatest reduction of HCV RNA. However, bodyweights of patients in the present study were 62 kg compared to 79–91 kg among US and European patients who were treated with boceprevir, PEG IFN and RBV combination therapy.[26] In fact, RBV dose-reduction rates and discontinuation rates of TVR treatment due to severe adverse events are higher in Japan than in the USA and Europe Union.[4–8] It was reported that the anti-HCV effect of triple therapy was similar when patients were given TVR at 1500 mg/day

compared with those given at 2250 mg/day in the Japanese patients.[27,28]

We demonstrated in this study that SVR may be anticipated in patients with the IFNL4 TT/TT genotype who are either treatment-naïve or who experienced relapse instead of non-response during prior IFN therapy. However, even among these patients, prediction of SVR is not assured. Furthermore, we are unable to predict the outcome of TVR triple therapy using only pretreatment predictors in patients who were previous non-responders, especially among those with unfavorable IFNL4 genotypes, until we can observe whether the patient achieves RVR. More accurate pretreatment prediction of the response is needed, and new treatments should be developed to ensure adequate treatment of all patients with chronic HCV infection, while avoiding the severe side-effects of TVR triple therapy.

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PNPLA3 I148M associations with liver carcinogenesis in Japanese chronic hepatitis C patients

Kazunori Nakaoka[1], Senju Hashimoto[1], Naoto Kawabe[1], Yoshifumi Nitta[1], Michihito Murao[1], Takuji Nakano[1], Hiroaki Shimazaki[1], Toshiki Kan[1], Yuka Takagawa[1], Masashi Ohki[1], Takamitsu Kurashita[1], Tomoki Takamura[1], Toru Nishikawa[1], Naohiro Ichino[2], Keisuke Osakabe[2] and Kentaro Yoshioka[1*]

Abstract

Aim: To investigate associations between patatin-like phospholipase domain-containing 3 (PNPLA3) genotypes and fibrosis and hepatocarcinogenesis in Japanese chronic hepatitis C (CHC) patients.

Methods: Two hundred and thirty-one patients with CHC were examined for PNPLA3 genotypes, liver stiffness measurements (LSM), and hepatocellular carcinoma (HCC) from May 2010 to October 2012 at Fujita Health University Hospital. The rs738409 single nucleotide polymorphism (SNP) encoding for a functional PNPLA3 I148M protein variant was genotyped using a TaqMan predesigned SNP genotyping assay. LSM was determined as the velocity of a shear wave (Vs) with an acoustic radiation force impulse. Vs cut-off values for cirrhosis were set at 1.55 m/s. We excluded CHC patients with a sustained virological response or relapse after interferon treatment.

Results: PNPLA3 genotypes were CC, CG, and GG for 118, 72, and 41 patients, respectively. Multivariable logistic regression analysis selected older age (OR = 1.06; 95% CI: 1.03–1.09; $p < 0.0001$), higher body mass index (BMI) (OR= 1.12; 95% CI: 1.03–1.22; $p = 0.0082$), and PNPLA3 genotype GG (OR = 2.07; 95% CI: 0.97–4.42; $p = 0.0599$) as the factors independently associated with cirrhosis. When 137 patients without past history of interferon treatment were separately assessed, multivariable logistic regression analysis selected older age (OR = 1.05; 95% CI: 1.02–1.09; $p = 0.0034$), and PNPLA3 genotype GG (OR = 3.35; 95% CI: 1.13–9.91; $p = 0.0291$) as the factors independently associated with cirrhosis. Multivariable logistic regression analysis selected older age (OR = 1.12; 95% CI: 1.07–1.17; $p < 0.0001$), PNPLA3 genotype GG (OR = 2.62; 95% CI: 1.15–5.96; $p = 0.0218$), and male gender (OR = 1.83; 95% CI: 0.90–3.71); $p = 0.0936$) as the factors independently associated with HCC.

Conclusion: PNPLA3 genotype I148M is one of risk factors for developing HCC in Japanese CHC patients, and is one of risk factors for progress to cirrhosis in the patients without past history of interferon treatment.

Keywords: PNPLA3; HCC; Chronic hepatitis C; SNP; Cirrhosis; HCV

Background

It is estimated that 130–170 million people, approximately 2%–3% of the world's population, are infected with the hepatitis C virus (HCV) (Shepard et al. 2005). Of the >500,000 new cases of hepatocellular carcinoma (HCC) that occur each year, approximately 25% are attributable to HCV infection (Block et al. 2003). In Japan, there are an estimated 880,000 HCV carriers aged 16–69 years, and 33,000 deaths occurred each year because of HCC, 81% of which were attributed to HCV infection (Yoshizawa et al. 2006). Treatments for chronic hepatitis C (CHC) have improved and the sustained virological response (SVR) rate has increased to 73%–86% (Fried et al. 2013; Wada et al. 2014). However, HCC still occurs in a large number of HCV carriers. Therefore, the elucidation of factors associated with the

* Correspondence: kyoshiok@fujita-hu.ac.jp
[1]Department of Liver, Biliary Tract and Pancreas Diseases, Fujita Health University, 1-98 Dengakugakubo, Kutsukakecho, Toyoake, Aichi 470-1192, Japan
Full list of author information is available at the end of the article

development of HCC is still an important task to be continued.

The rs738409 single nucleotide polymorphism (SNP) encoding for a functional I148M protein variant of the patatin-like phospholipase domain-containing 3 (PNPLA3, adiponutrin) gene is associated with hepatic steatosis, inflammation, fibrosis, and carcinogenesis in nonalcoholic fatty liver disease (NAFLD) (Romeo et al. 2008; Rotman et al. 2010; Kawaguchi et al. 2012; Kitamoto et al. 2013). This PNPLA3 gene polymorphism has also been reported to be associated with hepatic steatosis, fibrosis, treatment response, and carcinogenesis with CHC (Valenti et al. 2011; Trepo et al. 2011; Cai et al. 2011; Valenti et al. 2012; Clark et al. 2012; Dunn et al. 2014; Ezzikouri et al. 2014; Moritou et al. 2013; Zampino et al. 2013; Trepo et al. 2014; Sato et al. 2013). However, several reports did not find an association of this PNPLA3 gene polymorphism and some pathological features in CHC (Trepo et al. 2011; Nischalke et al. 2011; Rembeck et al. 2012; Miyashita et al. 2012; Takeuchi et al. 2013; Nakamura et al. 2013; Guyot et al. 2013). Therefore, the association of this PNPLA3 gene polymorphism and pathological features remains to be validated.

Although liver biopsy is the gold standard for diagnosing liver fibrosis, it is an invasive procedure and incurs a high cost. Therefore, it is difficult to perform liver biopsies when numerous patients are involved. However, noninvasive methods have been developed to assess liver fibrosis. Liver stiffness measurements (LSM) by transient elastography (TE) with a Fibroscan (Arima et al. 2010) and the velocity of a shear wave (Vs) measured by an acoustic radiation force impulse (ARFI) (Friedrich-Rust et al. 2009; Nishikawa et al. 2014) are correlated with the liver fibrosis stage in various liver diseases.

In the present study, we investigated possible associations of a PNPLA3 gene polymorphism with fibrosis and the development of HCC in Japanese patients with CHC. We used ARFI to assess hepatic fibrosis.

Results

PNPLA3 genotypes

For PNPLA3 (rs738409 C > G) genotypes, 118 patients had CC, 72 had CG, and 41 had GG. The G allele frequency was 33.3%. Vs values tended to be higher for patients with GG than for those with CG (p = 0.0636) and were higher than for those with CC, although there was no statistically significant difference (Figure 1). The frequency of HCC was higher among patients with GG than among those with CG or CC, although there was no statistically significant difference (Figure 2). Therefore, subsequent comparisons were made between patients with GG and those with CG or CC.

The patients with GG tended to have higher aspartate aminotransferase (AST) levels (p = 0.0946) and higher

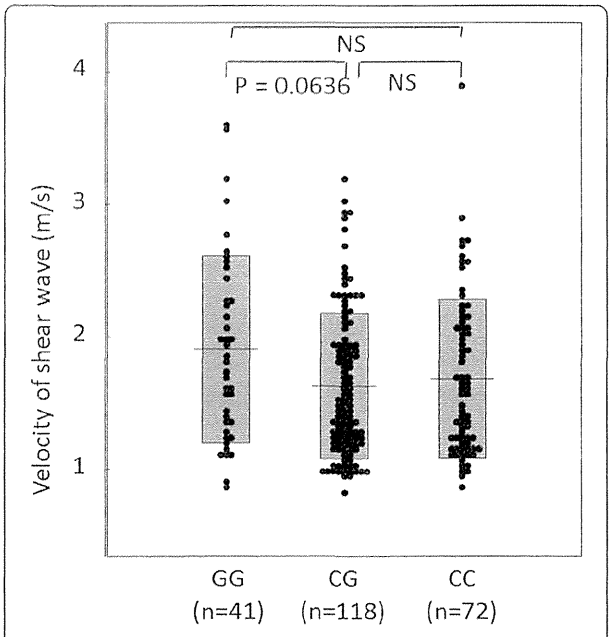

Figure 1 Vs values among PNPLA3 genotypes. Vs values tended to be higher for patients with GG than for those with CG (p = 0.0636) and was higher than for those with CC, although there was no statistically significant difference.

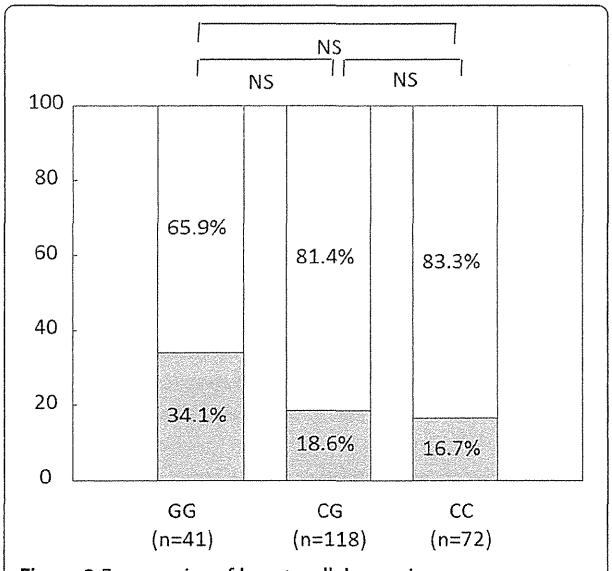

Figure 2 Frequencies of hepatocellular carcinoma among PNPLA3 genotypes. The hepatocellular carcinoma frequency was higher for patients with GG than for those with CG or for those with CC, although there was no statistically significant difference.

total bilirubin levels (p = 0.0876) and had significantly lower platelet counts (p = 0.0276), lower prothrombin times (p = 0.0407), higher hyaluronic acid levels (p = 0.0365), higher Vs values (p = 0.0126), and a higher frequency of HCC (p = 0.0200) than those with CG or CC (Table 1).

Factors associated with cirrhosis estimated by ARFI

Vs cut-off values for cirrhosis were set at 1.55 m/s, based on a report by Sporea et al.(Sporea et al. 2012). A total of 117 patients had Vs values of ≥ 1.55 m/s and were considered to have liver cirrhosis. As shown in Table 2, cirrhosis was associated with older age (p < 0.0001), higher body mass index (BMI) values (p = 0.0281), PNPLA3 genotype GG (p = 0.0318), higher AST levels (p < 0.0001), higher alanine aminotransferase (ALT) levels (p = 0.0195), lower albumin levels (p < 0.0001), lower platelet counts (p < 0.0001), lower prothrombin times (p < 0.0001), higher hyaluronic acid levels (p < 0.0001), higher α-fetoprotein (AFP) levels (p = 0.0452), higher protein induced by the vitamin K absence or antagonist-II (PIVKA-II) levels (p = 0.0023), HCV genotype 1 (p = 0.0283), and the presence of HCC (p < 0.0001).

Factors possibly associated with the progression to cirrhosis were assessed by multivariable regression analysis (Table 2). These factors included age, gender, BMI, PNPLA3 genotype, and HCV genotype. AST, ALT, albumin, platelet count, prothrombin time, hyaluronic acid, AFP, and PIVKA-II that were associated with cirrhosis by univariate analyses were excluded because they were apparently the result of cirrhosis but not the causes for the progression to cirrhosis. Because gender was reported to be associated with progression to cirrhosis (Poynard et al. 1997), gender, which was not associated with cirrhosis by univariate analysis, was included among the factors possibly associated with progression to cirrhosis. This analysis showed that older age (OR = 1.06; 95% CI: 1.03–1.09; p < 0.0001), higher BMI values (OR = 1.12; 95% CI: 1.03–1.22; p = 0.0082), and PNPLA3 genotype GG (OR = 2.07; 95% CI: 0.97–4.42; p = 0.0599) were factors independently associated with progression to cirrhosis, although the association with PNPLA3 genotype GG was only a tendency.

One hundred thirty seven patients without past history of interferon (IFN) treatment were separately assessed. Cirrhosis was associated with age (p = 0.0011), PNPLA3 genotype (p = 0.0113), AST levels (p < 0.0001), ALT levels (p = 0.0027), albumin levels (p < 0.0001), total bilirubin levels (p = 0.0078), platelet counts (p < 0.0001), prothrombin times (p = 0.0002), hyaluronic acid levels

Table 1 Characteristics of 231 patients studied and comparison among PNPLA3 genotypes

	All patients (n = 231)	Patients with GG (n = 41)	Patients with CC or CG (n = 190)	Comparison between patients with GG and those with CG or CG
Age (yrs)	62.9 ± 11.3	63.8 ± 10.6	62.7 ± 11.5	NS
Gender (male/female)	103/128	19/22	84/106	NS
BMI (kg/m^2)	22.5 ± 3.5	23.3 ± 3.6	22.3 ± 3.5	NS
Response to IFN treatment (NVR/no past IFN therapy)	94/137	19/22	75/115	NS
PNPLA3 (GG/CG/CC)	41/118/72			
AST (IU/L)	57.0 ± 48.8	68.5 ± 61.9	54.5 ± 45.3	p = 0.0946
ALT (IU/L)	62.9 ± 76.1	63.8 ± 51.6	62.7 ± 80.5	NS
γ-GTP (IU/L)	58.9 ± 78.1	60.6 ± 63.5	58.5 ± 81.0	NS
Albumin (g/dL)	4.1 ± 0.6	4.0 ± 0.6	4.1 ± 0.6	NS
Total bilirubin (mg/dL)	1.0 ± 0.8	1.2 ± 0.8	0.9 ± 0.8	p = 0.0876
Platelet count (x10^4/μL)	13.4 ± 5.6	11.7 ± 5.4	13.8 ± 5.5	p = 0.0276
Prothrombin time (%)	94.3 ± 18.6	88.9 ± 17.7	95.5 ± 18.7	p = 0.0407
Hyaluronic acid (ng/mL)	236.6 ± 337.7	340.5 ± 490.3	214.5 ± 292.3	p = 0.0365
α-fetoprotein (ng/mL)	79.1 ± 529.3	66.3 ± 193.5	81.9 ± 578.0	NS
PIVKA-II(mAU/mL)	24.7 ± 23.1	29.3 ± 36.4	23.7 ± 18.9	NS
HCV genotype (1/2/3)	188/41/2	34/7/0	150/37/2	NS
HCV RNA (log IU/mL)	6.1 ± 1.0	6.1 ± 1.3	6.1 ± 1.0	NS
Velocity of shear wave (m/s)	1.66 ± 0.52	1.91 ± 0.70	1.65 ± 0.57	p = 0.0126
Hepatocellular carcnoma (present/absent)	48/183	14/27	22/96	p = 0.0200

PNPLA3, patatin-like phospholipase domain-containing 3; BMI, body mass index; IFN, interferon; NVR, non-virological response; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyltranspeptidase; PIVKA-II, protein induced by Vitamin K absence or antagonist-II; Vs, velocity of shear wave; NS, not significant.

Table 2 Comparison between the patients with Vs < 1.55 m/s and those with Vs ≧ 1.55 m/s in all the 231 patients

	Patients with Vs ≧ 1.55 m/s (n = 117)	Patients with Vs < 1.55 m/s (n = 114)	Comparison between patients with Vs < 1.55 m/s and those with Vs ≧ 1.55 m/s	Multiple regression analysis for factors associated with ≧ 1.55 m/s	
				Odds ratio (95% confidence interval)	p
Age (yrs)	66.1 ± 10.0	60.0 ± 11.6	p < 0.0001	1.06 (1.03 - 1.09)	p < 0.0001
Gender (male/female)	51/66	52/62	NS		NS
BMI (kg/m^2)	23.0 ± 3.7	22.0 ± 3.2	p = 0.0281	1.12 (1.03 - 1.22)	p = 0.0082
Response to IFN treatment (NVR/no past IFN therapy)	45/72	49/65	NS		
PNPLA3 (GG/CC・CG)	27/90	14/100	p = 0.0318	2.07 (0.97 - 4.42)	p = 0.0599
AST (IU/L)	70.2 ± 47.3	42.4 ± 46.4	p < 0.0001		
ALT (IU/L)	74.0 ± 79.0	50.64 ± 71.3	p = 0.0195		
γ-GTP (IU/L)	61.2 ± 50.2	56.4 ± 100.4	NS		
Albumin (g/dL)	3.8 ± 0.7	4.4 ± 0.4	p < 0.0001		
Total bilirubin (mg/dL)	1.03 ± 0.56	0.87 ± 1.05	NS		
Platelet count (x10^4/μL)	10.7 ± 4.4	16.3 ± 5.2	p < 0.0001		
Prothrombin time (%)	88.0 ± 14.8	101.2 ± 20.0	p < 0.0001		
Hyaluronic acid (ng/mL)	348.1 ± 330.7	123.0 ± 306.7	p < 0.0001		
α-fetoprotein (ng/mL	146.0 ± 726.0	5.30 ± 5.2	p = 0.0452		
PIVKA-II(mAU/mL)	29.3 ± 29.8	20.0 ± 10.9	p = 0.0023		
HCV genotype (1/2/3)	101/14/2	87/27/0	p = 0.0283		NS
HCV RNA (log IU/mL)	6.2 ± 0.9	6.1 ± 1.1	NS		
Velocity of shear wave (m/s)	1.98 ± 0.46	1.27 ± 0.26	p < 0.0001		
Hepatocellular carcnoma (present/absent)	42/75	6/108	p < 0.0001		

Vs, velocity of shear wave; BMI, body mass index; IFN, interferon; NVR, non-virological response; PNPLA3, patatin-like phospholipase domain-containing 3; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyltranspeptidase; PIVKA-II, protein induced by Vitamin K absence or antagonist-II ; NS, not significant.

(p = 0.0006), AFP levels (p = 0.0553), PIVKA-II levels (p = 0.0072), and the presence of HCC (p < 0.0001) (Table 3). Age, gender, and PNPLA3 genotype were assessed for the factors possibly associated with the progression to cirrhosis by multivariable regression analysis (Table 3). This analysis showed that older age (OR = 1.05; 95% CI: 1.02–1.09; p = 0.0034), and PNPLA3 genotype GG (OR = 3.35; 95% CI: 1.13–9.91; p = 0.0291) were factors independently associated with progression to cirrhosis.

Ninety four patients with non-virological response (NVR) of past IFN treatment were separately assessed. Cirrhosis was associated with age (p = 0.0026), BMI values (p = 0.0274), albumin levels (p < 0.0001), platelet counts (p < 0.0001), prothrombin times (p < 0.0001), hyaluronic acid levels (p < 0.0001), AFP levels (p < 0.0001), and the presence of HCC (p = 0.0017) (Table 4). Age, gender, and BMI were assessed for the factors possibly associated with the progression to cirrhosis by multivariable regression analysis (Table 4). This analysis showed that older age (OR = 1.08; 95% CI: 1.03–1.13; p = 0.0023), and higher BMI values (OR = 1.20; 95% CI: 1.04–1.39; p = 0.0156) were factors independently associated with progression to cirrhosis.

Factors associated with the development of HCC

As shown in Table 5, HCC was associated with older age (p < 0.0001), PNPLA3 genotype GG (p = 0.0200), higher AST levels (p = 0.0158), lower albumin levels (p < 0.0001), higher total bilirubin levels (p = 0.0010), lower platelet counts (p < 0.0001), lower prothrombin times (p < 0.0001), higher hyaluronic acid levels (p < 0.0001), higher AFP levels (p = 0.0009), higher PIVKA-II levels (p = 0.0030), and higher Vs values (p < 0.0001).

Factors possibly associated with the development of HCC were assessed by multivariable regression analysis (Table 5). These factors included age, gender, and PNPLA3 genotype. AST, albumin, total bilirubin, platelet count, prothrombin time, hyaluronic acid, AFP, PIVKA-II, and Vs that were associated with HCC by univariate analyses were excluded because they were apparently the result of cirrhosis, which is a major risk factor for HCC,

Table 3 Comparison between the patients with Vs < 1.55 m/s and those with Vs ≧ 1.55 m/s in the 137 patients without past history of IFN treatment

	Patients with Vs ≧ 1.55 m/s (n = 72)	Patients with Vs < 1.55 m/s (n = 65)	Comparison between patients with Vs < 1.55 m/s and those with Vs ≧ 1.55 m/s	Multiple regression analysis for factors associated with ≧ 1.55 m/s	
				Odds ratio (95% confidence interval)	p
Age (yrs)	67.3±10.3	60.9±12.0	P = 0.0011	1.05 (1.02 - 1.09)	p = 0.0034
Gender (male/female)	33/39	31/34	NS		NS
BMI (kg/m^2)	22.6±3.6	21.7±3.4	NS		
PNPLA3 (GG/CC • CG)	17/55	5/60	P = 0.0113	3.35 (1.13 - 9.91)	p = 0.0291
AST (IU/L)	76.7±56.2	37.3±28.4	p < 0.0001		
ALT (IU/L)	82.9±96.2	43.6±41.1	P = 0.0027		
γ-GTP (IU/L)	64.0±57.3	51.9±77.1	NS		
Albumin (g/dL)	3.7±0.6	4.3±0.4	p < 0.0001		
Total bilirubin (mg/dL)	1.1±0.7	0.8±0.6	P = 0.0078		
Platelet count (x10^4/μL)	10.4±4.5	15.9±5.3	p < 0.0001		
Prothrombin time (%)	87.1±15.4	99.1±21.5	P = 0.0002		
Hyaluronic acid (ng/mL)	385.0±383.0	148.8±371.4	P = 0.0006		
α-fetoprotein (ng/mL)	233.9±939.1	6.8±10.8	P = 0.0553		
PIVKA-II(mAU/mL)	32.9±36.1	20.0±11.9	P = 0.0072		
HCV genotype (1/2/3)	61/10/1	48/17/0	NS		
HCV RNA (log IU/mL)	6.0±0.9	6.2±1.3	NS		
Velocity of shear wave (m/s)	2.14±0.46	1.23±1.0	p < 0.0001		
Hepatocellular carcnoma (present/absent)	28/44	3/62	p < 0.0001		

Vs, velocity of shear wave; BMI, body mass index; IFN, interferon; PNPLA3, patatin-like phospholipase domain-containing 3; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyltranspeptidase; PIVKA-II, protein induced by Vitamin K absence or antagonist-II ; NS, not significant.

but not the causes of cirrhosis or development of HCC. Because gender was reported to be associated with the development of HCC (Asahina et al. 2010), gender, which was not associated with HCC by univariate analysis, was included for multivariable analysis. This analysis showed that older age (OR = 1.12; 95% CI: 1.07–1.17; p < 0.0001), PNPLA3 genotype GG (OR = 2.62; 95% CI: 1.15–5.96; p = 0.0218), and male gender (OR = 1.83; 95% CI: 0.90–3.71; p = 0.0936) were factors independently associated with the development of HCC, although the association with gender was only a tendency.

One hundred thirty seven patients without past history of IFN treatment were separately assessed. HCC was associated with age (p = 0.0002), PNPLA3 genotype (p = 0.0928), AST levels (p = 0.0032), albumin levels (p < 0.0001), total bilirubin levels (p < 0.0001), platelet counts (p = 0.0008), prothrombin times (p = 0.0002), hyaluronic acid levels (p = 0.0002), AFP levels (p = 0.0016), PIVKA-II levels (p < 0.0001), and Vs values (p < 0.0001) (Table 6). Age, gender, and PNPLA3 genotype were assessed for factors possibly associated with the development of HCC by multivariable regression analysis (Table 6). This analysis showed that older age (OR = 1.09; 95% CI: 1.04–1.15; p = 0.0006) was an only factor independently associated with the development of HCC.

Ninety four patients with NVR of past IFN treatment were separately assessed. HCC was associated with age (p < 0.0001), PNPLA3 genotype (p = 0.0871), albumin levels (p < 0.0001), platelet counts (p = 0.0008), prothrombin times (p = 0.0295), hyaluronic acid levels (p = 0.0002), AFP levels (p = 0.0005), PIVKA-II levels (p = 0.0134), and Vs values (p = 0.0002) (Table 7). Age, gender, and PNPLA3 genotype were assessed for the factors possibly associated with the development of HCC by multivariable regression analysis (Table 7). This analysis showed that older age (OR = 1.19; 95% CI: 1.08–1.32; p = 0.0007), and PNPLA3 genotype GG (OR = 3.95; 95% CI: 1.00–15.61; p = 0.0497) were factors independently associated with the development of HCC.

Discussion

In this study, we demonstrated that a PNPLA3 gene polymorphism was associated with the progression of fibrosis to cirrhosis and development of HCC, although the association with cirrhosis was only a tendency by multivariable analysis in all the 231 patients studied.

Table 4 Comparison between the patients with Vs < 1.55 m/s and those with Vs ≧ 1.55 m/s in the 94 patients with NVR of past IFN treatment

	Patients with Vs ≧ 1.55 m/s (n = 45)	Patients with Vs < 1.55 m/s (n = 49)	Comparison between patients with Vs < 1.55 m/s and those with Vs ≧ 1.55 m/s	Multiple regression analysis for factors associated with ≧ 1.55 m/s	
				Odds ratio (95% confidence interval)	p
Age (yrs)	64.3±9.4	57.7±10.9	P = 0.0026	1.03 (1.03 - 1.13)	p = 0.0023
Gender (male/female)	18/27	21/28	NS		NS
BMI (kg/m^2)	23.7±3.8	22.2±2.8	P = 0.0274	1.20 (1.04 - 1.39)	p = 0.0156
PNPLA3 (GG/CC・CG)	10/35	9/40	NS		
AST (IU/L)	59.6±22.0	51.7±63.9	NS		
ALT (IU/L)	58.6±31.2	62.8±98.8	NS		
γ-GTP (IU/L)	57.2±37.1	62.1±122.7	NS		
Albumin (g/dL)	3.9±0.7	4.4±0.3	p < 0.0001		
Total bilirubin (mg/dL)	0.9±0.3	1.0±1.4	NS		
Platelet count (x10^4/μL)	11.1±4.4	16.7±5.1	p < 0.0001		
Prothrombin time (%)	88.5±14.1	104.0±16.5	p < 0.0001		
Hyaluronic acid (ng/mL)	305.2±229.7	89.3±170.5	p < 0.0001		
α-fetoprotein (ng/mL)	20.1±18.0	4.9±3.6	p < 0.0001		
PIVKA-II(mAU/mL)	23.5±13.9	19.9±9.3	NS		
HCV genotype (1/2/3)	40/4/1	39/10/0	NS		
HCV RNA (log IU/mL)	6.3±0.9	6.0±1.3	NS		
Velocity of shear wave (m/s)	2.20±0.50	1.23±0.16	p < 0.0001		
Hepatocellular carcnoma (present/absent)	14/31	3/46	P = 0.0017		

NVR, non-virological response; IFN, interferon; Vs, velocity of shear wave; BMI, body mass index; PNPLA3, patatin-like phospholipase domain-containing 3; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyltranspeptidase; PIVKA-II, protein induced by Vitamin K absence or antagonist-II ; NS, not significant.

When the patients without past IFN treatment and those with NVR of past IFN treatment were separately analyzed, a PNPLA3 gene polymorphism was selected as a factor independently associated with progression to cirrhosis in those without previous IFN treatment but not in those with NVR of past IFN treatment. A PNPLA3 gene polymorphism was selected as a factor independently associated with development to HCC in those with NVR of past IFN treatment but not in those without past IFN treatment.

PNPLA3 polymorphisms have been reported to be associated with hepatic steatosis, inflammation, fibrosis, and carcinogenesis in NAFLD (Romeo et al. 2008; Rotman et al. 2010; Valenti et al. 2010; Sookoian and Pirola 2011; Burza et al. 2012; Kawaguchi et al. 2012; Kitamoto et al. 2013). PNPLA3 polymorphisms have also been reported to be associated with hepatic steatosis, fibrosis, treatment response, and carcinogenesis in CHC (Valenti et al. 2011; Trepo et al. 2011; Cai et al. 2011; Valenti et al. 2012; Clark et al. 2012; Dunn et al. 2014; Ezzikouri et al. 2014; Moritou et al. 2013; Zampino et al. 2013; Trepo et al. 2014; Sato et al. 2013). However, several reports have not found an association of PNPLA3 polymorphisms with fibrosis and carcinogenesis in CHC (Trepo et al. 2011; Nischalke et al. 2011; Rembeck et al. 2012; Miyashita et al. 2012; Takeuchi et al. 2013; Nakamura et al. 2013; Guyot et al. 2013).

A significant association was reported between a PNPLA3 polymorphism and HCC in patients with CHC (Valenti et al. 2011; Ezzikouri et al. 2014), while other studies did not find a significant association(Nischalke et al. 2011; Guyot et al. 2013). A meta-analysis performed by Trepo et al. showed that a PNPLA3 polymorphism was strongly associated with HCC, although the association was stronger in patients with alcoholic liver disease (OR = 2.20; 95% CI: 1.802.67; P = 4.71 × 10^{-15}) than that in patients with CHC (OR = 1.55; 95% CI: 1.03–2.34; P = 3.52 × 10^{-2}) (Trepo et al. 2014). In Japanese studies, Moritou et al. reported that a PNPLA3 polymorphism was significantly associated with serum AFP level (Moritou et al. 2013), and Sato et al. reported that the median time between HCV infection and the development of HCC was significantly shorter for patients with the PNPLA3 GG genotype in HCV-related HCC (Sato et al. 2013). The