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FORUM

肝炎をめぐる医療政策

正木尚彦／まさきなおひこ
国立国際医療研究センター 肝炎・免疫研究センター 肝炎情報センター

わが国には肝炎ウイルスキャリアが約350万人存在すると推定されており，その内訳はB型肝炎110～140万人，C型肝炎190～230万人である．まさに“ウイルス肝炎は国民病である”との認識が妥当であり，国の医療政策の原点となっている．とくに2010年1月に“肝炎対策基本法”が施行されたことにより，現行の肝炎総合対策に対してこれまで以上の俊敏さ，具体的実効性が求められている．

■肝炎対策基本法

さて，この法律の前文には「B型肝炎及びC型肝炎に係るウイルスへの感染については，国の責めに帰すべき事由によりもたらされ，又はその原因が解明されていなかったことによりもたらされたものがある．特定の血液凝固因子製剤にC型肝炎ウイルスが混入することによって不特定多数の者に感染被害を出した薬害肝炎事件では，感染被害者の方々に甚大な被害が生じ，その被害の拡大を防止し得なかったことについて国が責任を認め，集団予防接種の際の注射器の連続使用によってB型肝炎ウイルスの感染被害を出した予防接種禍事件では，最終の司法判断において国の責任が確定している（下線は著者による追加）」と明記されていることからわかるように，ウイルス肝炎蔓延の原因の一部にわが国固有の事案が存在する．誌幅の都合上，詳細は割愛するが，薬害肝炎事件は凝固因子製剤（フィブリノゲン，第Ⅸ因子）へのC型肝炎ウイルスの混入に起因し（推定患者数1万人以上），一方，予防接種禍事件は，集団予防接種など（予防接種およびツベルクリン反応検査）の際の注射器の連続使用によってB型肝炎ウイルスの水平感染を招いたとされる事案（推定患者数40万人以上）である．前者では患者・国・製薬会社の3者間，後者では患者・国の2者間での和解が成立し，補償が進められているところである．

■都道府県肝疾患診療ネットワークの構築

国がこれまで行ってきたさまざまな肝炎対策のなかで，もっとも画期的な施策の一つが2002～2006年度の5年間全国で展開された節目検診，節目外検診である．B型肝炎ウイルス検診受診者はのべ8,704,587人で，うち100,983人（1.16%）が“陽性”と判定された．一方，C型肝炎ウイルス検診

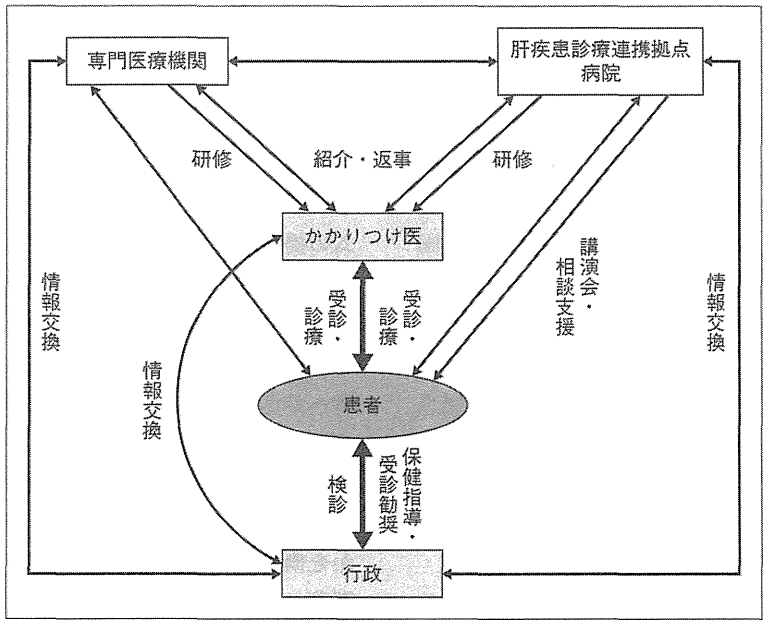

図1 都道府県における肝疾患診療ネットワーク構築（2007年1月厚生労働省）

表 1　肝疾患診療連携拠点病院，専門医療機関に必要とされる資格要件

肝疾患診療連携拠点病院
①肝疾患診療にかかわる一般的な医療情報の提供
②都道府県内の専門医療機関等に関する情報の収集や紹介
③医療従事者や地域住民を対象とした研修会や講演会の開催や肝疾患に関する相談支援
④肝疾患に関する相談医療機関と協議の場の設定
専門医療機関
①専門的な知識をもつ医師による診断と治療方針の決定が可能
②インターフェロンなどの抗ウイルス療法が可能
③肝癌の高危険群の同定と早期診断が可能

受診者はのべ8,634,509人に達し，うち99,950人(1.16%)が“現在，C型肝炎ウイルスに感染している可能性がきわめて高い”と判定された．しかし，その結果が検診受診者に通知されたにもかかわらず，二次精検を目的とした医療機関への受診率は3〜4割程度にとどまり，インターフェロン療法などの抗ウイルス療法を受けた患者数も当初の期待に遠く及ばなかったと推定された．さらに，全国津々浦々における肝疾患診療体制がかならずしも整備されていないという状況も指摘されていた．これを改善するために，国は2007年1月に“都道府県における肝炎検査後肝疾患診療体制に関するガイドライン”を発出し，各都道府県において“かかりつけ医と患者の最小単位”を支援する診療ネットワークを行政側，医療側含めて構築することとした(図1)．この施策に基づいて，自治体ごとに原則1カ所の肝疾患診療連携拠点病院，二次医療圏ごとに肝疾患専門医療機関の指定が進められてきた．これらの施設指定に必要な資格要件を表1に示す．2011年4月1日現在，肝疾患診療ネットワークの要である肝疾患診療連携拠点病院の指定がようやく47都道府県で完了し，全国で70病院となっている．その内訳をみると，国立大学法人が34病院，公立・私立大学が24病院，その他(国立病院機構，県立病院，一般病院など)が12病院となっている．なお，肝疾患患者数が多く広域に分布しているなどの理由で，複数の拠点病院を指定している自治体もある(国立国際医療研究センター肝炎情報センターホームページ：http://www.ncgm.go.jp/center/index.html参照)．

■肝炎情報センターの果たすべき役割

さらに，都道府県単位の活動を支援するシステムとして，国立国際医療センター(現国立国際医療研究センター)に2008年11月，肝炎情報センターが設置された(千葉県市川市)．その果たすべき役割として3つのミッションがある[1]．

第1に“インターネットなどによる最新情報提供”であり，2008年12月には肝疾患医療に関する診療ガイドライン，肝炎診療をめぐる国内外の情報などを“一般向け，医療従事者向け，および肝臓専門医向け”に発信するためのホームページを立ち上げた．第2に“拠点病院間での情報共有を支援する”ことで，肝疾患診療連携拠点病院で構成する連絡協議会を年に2回開催し，拠点病院事業における問題点の解決をめざした話し合いを行っている．第3に，肝疾患診療連携拠点病院などに勤務する医療従事者(医師，看護師，相談員，臨床検査技師ほか)を対象とした“研修会”の企画・立案・推進を行っている．とくに，拠点病院事業のひとつである肝疾患相談センターの運営にとって必要不可欠な相談員の育成は最重要課題として位置づけており，相談員が患者からのさまざまな問い合わせに対応できるように，医療資源の活用法に関する知識の習得，患者とのコミュニケーションスキルの向上をめざした研修プログラムの提供をはかっている．

■肝疾患患者に対する医療費助成事業

国と都道府県が共同で行う施策には，肝疾患患者を取り巻く医療環境の整備のほかに，肝疾患患者への治療促進を目的とした医療費助成事業がある．その実施主体は各都道府県であり，財源負担は国：地方＝1：1である．肝炎治療に1カ月分(3割負担)としてどれくらいの薬剤費が必要であるかを概算すると，B型肝炎に対する核酸アナログ製剤の1日分薬価がラミブジン，アデホビル，エンテカビルそれぞれ，622.00円，1,252.10円，

1,032.30円であることから，ラミブジン耐性患者でラミブジン・ヘプセラを併用すると1,874.1×28×0.3＝15,742円，エンテカビル単独で1,032.3×28×0.3＝8,671円となる．一方，C型慢性肝炎の標準的治療であるペグインターフェロン・リバビリン併用療法については，ペグイントロン100 μg注29,550.0円，レベトール200 mgカプセル764.60円であることから，体重65 kgとして1カ月分(3割負担)で(29,550×4＋764.6×4×28)×0.3＝61,151円となる．ペガシス・コペガス併用療法の場合も，ほぼ同額である．さらに，2011年9月に保険承認されたテラプレビル(テラビック®)も1錠1,422.1円と非常に高額で，1日分(9錠)が1,422.1×9×0.3＝3,840円のため，12週間3剤併用＋12週間2剤の24週間治療で3割負担の場合，約68.9万円(1カ月分11.5万円)に達する．したがって，抗ウイルス療法を広く普及させるためには，医療費助成がきわめて有効と考えられる．このような観点から，国と都道府県は肝炎治療特別促進事業として，2008年度からはB型・C型ウイルス性肝炎に対するインターフェロン治療，2010年度からはB型肝炎に対する核酸アナログ製剤治療への医療費助成を開始している．自己負担限度額は所得に応じて当初は1万円・3万円・5万円であったが，その後，1万円・2万円とさらなる負担軽減がはかられている．テラプレビルについても，医療費助成の対象となることが2011年11月28日付でいち早く決定された．これとは別に，身体障害者認定による重度肝硬変患者への医療費助成が2010年度から開始されている．対象者は，肝硬変の重症度分類として繁用されているChild-Pugh分類の合計点数10点以上(グレードCに該当)が3カ月以上持続していることが前提で，加えて日常生活活動の制限などに関する項目数などに応じて，もっとも障害程度の重い1級から，もっとも軽症な4級までの4等級に分類されている．肝硬変の原因として肝炎ウイルスに起因するもの以外も含まれているが，とくにアルコールに起因するものについては，6カ月以上の禁酒の確認が厳しく求められている．さらに，肝移植とこれに伴う医療も自立支援医療の対象とされており，医療費の自己負担額軽減がはかられている．とくに，移植後に抗免疫療法を必要とする期間は，これを実施しないと肝機能が廃絶する危険性があるため，障害程度1級と認定される．

■おわりに

冒頭で述べたように，現行の肝炎総合対策は“肝炎対策基本法”という法律に基づいて進められている．第一章第一条において「国，地方公共団体，医療保険者，国民及び医師等の責務を明らかにし，……」と述べられているように，国民すべてに担うべき役割があることを認識すべきである．

文献

1) 正木尚彦：肝炎情報センターの役割．肝・胆・膵，**61**(5)：721-729，2010.

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ORIGINAL ARTICLE

Estimation of two real-time RT-PCR assays for quantitation of hepatitis C virus RNA during PEG-IFN plus ribavirin therapy by HCV genotypes and IL28B genotype

Yugo Miyagi · Hideyuki Nomura · Nobuyuki Yamashita · Hironori Tanimoto · Kiyoaki Ito · Naohiko Masaki · Masashi Mizokami · Tsunefumi Shibuya

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Abstract Hepatitis C virus (HCV) RNA values measured with two real-time PCR methods (Cobas Ampliprep/Cobas TaqMan, CAP/CTM, and the Abbott real-time PCR test, ART) vary among patients with genotype 1. We investigated HCV RNA values measured by two real-time PCR assays during pegylated interferon plus ribavirin (PEG-IFN/RBV) therapy. We evaluated 185 cases of chronic hepatitis C patients, among which 97 patients received the PEG-IFN/RBV therapy. HCV RNA values of CAP/CTM for genotype 1 were significantly higher than those of ART ($p < 0.05$) The difference in HCV RNA values (CAP/CTM minus ART) of genotype 1 was significantly higher than those in genotype 2 ($p < 0.0001$). The positive rate (>0) of the difference of HCV RNA values in genotype 1 was 100 % (55/55), which was significantly higher than the 78.6 % (33/42) of genotype 2 ($p < 0.001$). There was no difference between TT and TG/GG genotype groups in terms of difference of HCV RNA values (CAP/CTM minus ART). After PEG-IFN/RBV therapy was administered, reduction of HCV measurements was observed from day 1 for both assays regardless of genotype. The HCV value of CAP/CTM during PEG-IFN/RBV therapy was consistently higher than the value of ART, although the difference in these two values gradually became smaller during the course of therapy, and eventually no significant difference was observed near the detection level. No correlation was observed between the sustained virological response (SVR) rate and the difference between the CAP/CTM HCV values and the ART HCV value before treatment.

Keywords Abbott real-time PCR test · Cobas Ampliprep/Cobas TaqMan · Hepatitis C virus · Genotype · PEG-IFN plus ribavirin therapy · Real-time RT-PCR assay

Introduction

Approximately 80 % of patients infected by hepatitis C virus (HCV) develop chronic hepatitis [1, 2]. Currently, there are more than 100 million HCV carriers worldwide. Chronic hepatitis C could gradually progress to cirrhosis and liver cancer. The first treatment option for chronic hepatitis C is the pegylated interferon plus ribavirin (PEG-IFN/RBV) combination therapy [3, 4]. Several virological predictive factors for sustained virological response (SVR) of PEG-IFN/RBV combination therapy are HCV genotype, baseline viral loads, and early virological response [5–7]. The SVR rate of PEG-IFN/RBV therapy is approximately 50 % for genotype 1 and 80 % for genotype 2. HCV RNA monitoring early in PEG-IFN/RBV therapy is an important predictive factor for SVR for either genotype 1 or genotype 2 [8, 9]. Detection of HCV RNA during PEG-IFN/RBV therapy is important in determining the length of IFN treatment [10]. Currently, Cobas Ampliprep/Cobas TaqMan (CAP/CTM) and Abbott real-time PCR test (ART) are used for HCV RNA measurement. The HCV RNA value in genotype 1 measured by CAP/CTM assay was significantly higher than values by ART assay [11]. The HCV RNA

Y. Miyagi · H. Nomura (✉) · N. Yamashita · H. Tanimoto · T. Shibuya
Department of Internal Medicine, Shin-Kokura Hospital, 1-3-1 Kanada, Kokurakitaku, Kitakyushu, Fukuoka 803-8505, Japan
e-mail: h-nomura@shin-kokura.gr.jp

K. Ito · N. Masaki · M. Mizokami
The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, Japan

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value in genotype 1 was measured by two real-time polymerase chain reaction (PCR) methods in this study to investigate whether there is a significant difference in HCV RNA values during PEG-IFN/RBV therapy.

Materials and methods

Of patients with chronic hepatitis C who visited Shin-Kokura hospital from April 2009 to December 2010, 185 were enrolled in this study. Of these 185 patients, 92 subjects were male and 93 were female, 96 subjects were 60 years old or older, and 89 were younger than 60 years old. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki and was approved by the Institutional Review Board. Each patient gave informed consent before participating in this trial. Of the 185 subjects in the study, 97 patients received the PEG-IFNa-2b plus ribavirin combination therapy: 55 patients had genotype 1, and 42 patients had genotype 2. PEG-IFNa-2b (PEG-Intron; MSD, Tokyo, Japan) was injected subcutaneously at a median dose 1.5 μg/kg (range, 1.3–1.5 μg/kg) once a week. Ribavirin (Rebetol; MSD, Tokyo, Japan) was administered at 200–600 mg twice a day after breakfast and dinner (daily dose, 600–1,000 mg). Patients were considered to have an SVR if HCV RNA remained undetectable at 24 weeks after the completion of treatment. The SVR rate was evaluated separately in patients with genotype 1 and genotype 2. Fifty-two of 55 cases of genotype 1 that received PEG-IFN/RBV were evaluated because the treatment was discontinued in 3 patients. Forty-one of 42 cases of genotype 2 were evaluated; treatment was discontinued in 1 patient.

Two real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assays, CAP/CTM (Roche Molecular Systems, Pleasanton, CA, USA) and Abbott real-time HCV test (ART; Abbott Molecular, Abbott Park, IL, USA) were used for the quantitative measurement of HCV RNA concentrations before PEG-IFN/RBV treatment and at day 1, week 1, and week 2 during PEG-IFN/RBV treatment.

Abbott real-time HCV test

The Abbott real-time HCV test is based upon RT-PCR followed by real-time fluorescent detection of HCV RNA (RT-PCR assay). The assay has adopted the second international WHO standard for HCV RNA (code 96/798) for calibration. HCV RNA concentration is expressed in IU/ml. The ART assay has a lower limit of detection (LOD) of 12 IU/ml with a linear quantitation range of 12×10^7 IU/ml.

Cobas Ampliprep/Cobas TaqMan assay

The Cobas Ampliprep/Cobas TaqMan assay is based upon RT-PCR followed by real-time fluorescent detection of HCV RNA from 850 ml serum. CAP/CTM is standardized against the first WHO international standard for HCV RNA (code 96/798). HCV RNA concentration is reported in IU/ml. CAP/CTM assay has an LOD of 15 IU/ml with a linear quantitation range of 43–6.9×10^7 IU/ml.

We genotyped 115 patients for a single nucleotide polymorphism (SNP): rs8099917, an IL28B SNP previously reported to be associated with PEG-IFN/RBV therapy outcome. Samples were genotyped using the Illumina Human Hap 610-Quad Genotyping Bead Chip, with the Invader, or TaqMan assay, as described elsewhere [11–13].

Data analysis

Statistical analysis was performed using PASW Statistics, version 18 (SPSS) and R, version 2.11. Categorical data were analyzed using the chi-squared test and Fisher's exact tests, and continuous data were analyzed using the nonparametric Mann–Whitney U test. p values (two-tailed) <0.05 were considered statistically significant. Correlation coefficient (R) was assessed by the Spearman's correlation coefficient implemented in STATA software version 8.0 (Stata-Corp. LP, College Station, TX, USA).

Results

Figure 1 shows the correlation between the HCV RNA measurements obtained by the two real-time PCR assays: CAP/CTM versus ART in the study variables. A strong correlation was noted between the two real-time RT-PCR assays with an overall coefficient of correlation (R^2) of 0.8975 ($p < 0.0001$).

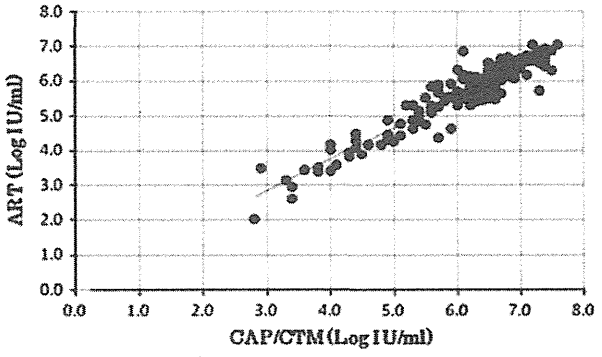

Fig. 1 Correlation between hepatitis C virus (HCV) RNA measurements obtained by two real-time RT-PCR assays: Cobas Ampliprep/Cobas TaqMan (*CAP/CTM*) versus Abbott real-time HCV test (*ART*)

Table 1 Correlation between hepatitis C (HCV) RNA measurements obtained by two real-time RT-PCR assays: CAP/CTM versus ART for study variables ($y = 0.9064x + 0.1176$, $R^2 = 0.8975$, $p < 0.0001$)

Study variables	*n*	CAP/CTM (log IU/ml)		ART (log IU/ml)		Correlation coefficient	
		Mean	SD	Mean	SD	R^2	*p* value
Gender							
Male	92	6.18	1.06	5.71	0.97	0.9009	<0.0001
Female	93	6.18	0.92	5.72	0.92	0.8972	<0.0001
Age (years)							
≥60	96	6.04	1.03	5.56	1.00	0.9142	<0.0001
<60	89	6.33	0.92	5.89	0.89	0.8709	<0.0001
Platelet counts (10^9/l)							
≥150	126	6.24	0.96	5.78	0.94	0.9106	<0.0001
<150	59	6.04	1.03	5.58	0.96	0.8705	<0.0001
IL28B							
TT	84	6.06	1.06	5.67	1.06	0.8826	<0.0001
TG/GG	31	5.91	1.18	5.45	1.07	0.9461	<0.0001
Genotype							
1	55	6.06	1.09	5.52	1.09	0.9647	<0.0001
2	42	5.94	1.21	5.65	1.15	0.9233	<0.0001

CAP/CTM Cobas Ampliprep/Cobas TaqMan, *ART* Abbott real-time HCV test, *IL28B* interleukin 28B

Table 1 shows the correlation between the HCV RNA measurements obtained by the two real-time RT-PCR assays, CAP/CTM versus ART, in the study variables. All the coefficient of correlation (R^2) values based on the variables, such as gender, age (≥60 or >60 years), and number of platelets (≤150 × 10^9/l or >150 × 10^9/l), were more than 0.8700 ($p < 0.0001$) and were strongly correlated with the HCV RNA values obtained by the two real-time RT-PCR assays. The coefficients of correlation (R^2) for IL28B genotype (TT, TG/GG) were 0.8826 ($p < 0.000$) and 0.9461 ($p < 0.0001$), respectively, and a strong correlation was observed also for the HCV RNA values obtained by the two real-time RT-PCR assays. Similarly, the coefficients of correlation (R^2) for the HCV genotypes (genotypes 1, 2) were 0.9647 ($p < 0.0001$) and 0.9233 ($p < 0.0001$), respectively, and a strong correlation was observed also for the HCV RNA values obtained by the two real-time PCR assays.

Table 2 shows HCV RNA concentrations of study variables as measured by the two real-time RT-PCR assays. HCV RNA values measured by CAP/CTM were significantly higher than those by ART for all variables, such as gender, age (≥60 or >60 years), and the number of platelets (≥150 × 10^9/l or >150 × 10^9/l) ($p < 0.05$). The HCV RNA values of the IL28B group with TT genotype measured by CAP/CTM were significantly higher than those by ART ($p < 0.05$); however, no difference was observed for the TG/GG genotypes. The difference of HCV RNA values (CAP/CTM minus ART) between the TT genotype and the TG/GG genotypes was not statistically significant (Fig. 2). The positive rates of the difference of HCV RNA values in the TT genotype and the TG/GG genotypes (CAP/CTM minus ART) were 90.5 and 90.3 %, respectively, and were not statistically significant. The difference of HCV RNA values in the TG/GG genotypes were not statistically significant, which can be explained by the small number of subjects enrolled in this study. The HCV RNA values in genotype 1 measured by CAP/CTM were significantly higher than those by ART ($p < 0.05$); however, the difference was not statistically significant in genotype 2. The difference of HCV RNA values (CAP/CTM minus ART) was significantly higher in genotype 1 than in genotype 2 ($p < 0.0001$) (Fig. 3). The positive rate (>0) of the difference of HCV RNA values (CAP/CTM minus ART) in genotype 1 was 100 % (55/55), significantly higher ($p < 0.001$) compared to the positive rate of 78.6 % (33/42) in genotype 2.

Table 3 shows HCV RNA concentrations of HCV genotypes 1 and 2 during PEG-IFN/RBV treatment as measured by the two real-time PCR assays. The HCV RNA values decreased during PEG-IFN/RBV therapy for both genotype 1 and genotype 2. The difference of HCV RNA values (CAP/CTM minus ART) in the genotype 1 group decreased gradually. The difference was not statistically significant at day 1 of treatment. The HCV RNA values of CAT/CTM in the genotype 1 group were higher than those of ART at day 1, week 1, and week 2; however, the difference was not statistically significant.

Table 4 shows the SVR rate in patients who received PEG-IFN/RBV therapy by differences of HCV RNA values between CAT/CTM and ART before PEG-IFN/RBV

Table 2 HCV RNA concentrations for study variables as measured by two real-time RT-PCR assays: CAP/CTM and ART

Study variables	*n*	CAP/CTM (log IU/ml)		ART (log IU/ml)		Average HCV RNA level (CAP/CTM−ART)		Quantitation difference		*p* value*
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Gender										
Male	92	6.18	1.06	5.71	0.97	5.95	1.00	0.47	0.33	0.0019
Female	93	6.18	0.92	5.72	0.92	5.95	0.91	0.45	0.30	0.0010
Age (years)										
≥60	96	6.04	1.03	5.56	1.00	5.80	1.00	0.48	0.30	0.0010
<60	89	6.33	0.92	5.89	0.89	6.11	0.87	0.44	0.33	0.0016
Platelet counts (10^9/l)										
≥150	126	6.24	0.96	5.78	0.94	6.01	0.94	0.46	0.29	0.0002
<150	59	6.04	1.03	5.58	0.96	5.81	0.98	0.47	0.37	0.0123
IL28B										
TT	84	6.06	1.06	5.67	1.06	5.86	1.04	0.40	0.34	0.0131
TG/GG	31	5.91	1.18	5.45	1.07	5.68	1.12	0.45	0.29	0.1199
Genotype										
1	55	6.06	1.09	5.52	1.09	5.00	1.09	0.54	0.21†	0.0161
2	42	5.94	1.21	5.65	1.15	5.79	1.17	0.28	0.34†	0.2734

CAP/CTM Cobas Ampliprep/Cobas TaqMan, *ART* Abbott real time HCV test, *IL28B* interleukin 28B

* CAP/CTM versus ART

† $p < 0.0001$, genotype 1 versus genotype 2

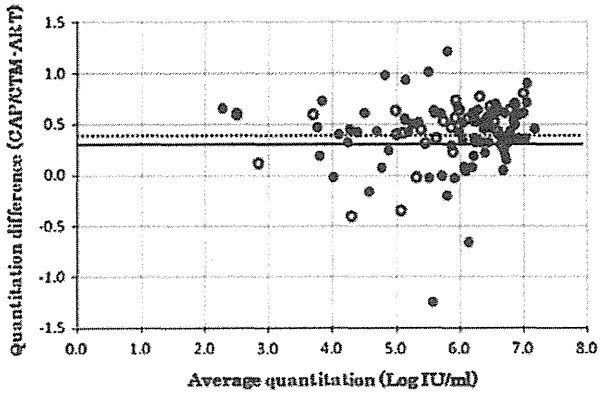

Fig. 2 Genotype-specific HCV RNA level difference in HCV RNA measurements by CAP/CTM versus those by ART test in samples with interleukin 28B (IL28B) genotypes; TT and TG/GG, before pegylated interferon plus ribavirin (PEG-IFN/RBV) treatment [*closed circles* genotype TT, *open circles* genotype TG/GG, *solid line* mean HCV RNA values of the difference (CAP/CTM minus ART) in TT, *dotted line* mean HCV RNA values of the difference (CAP/CTM minus ART) in TG/GG]

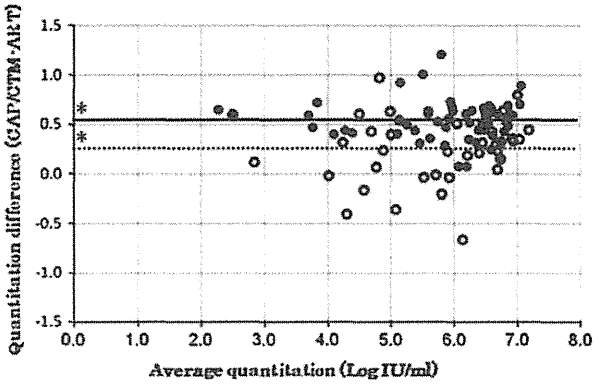

Fig. 3 Genotype-specific HCV RNA level difference in HCV RNA measurements by CAP/CTM versus those by ART test in samples with HCV subtypes 1 and 2 before PEG-IFN/RBV treatment [*closed circles* genotype 1, *open circles* genotype 2, *solid line* mean HCV RNA values of the difference (CAP/CTM minus ART) in HCV genotype 1, *dotted line* mean HCV RNA values of the difference (CAP/CTM minus ART) in HCV genotype 2]. *$p < 0.0001$, genotype 1 versus genotype 2

therapy. Group L comprises patients with a difference of HCV RNA values of 0.5 IU/ml or more (CAP/CTM minus ART), and group S comprises patients with a difference of HCV RNA values of less than 0.5 IU/ml (CAP/CTM minus ART). The SVR rate of genotype 1 (55.8 %) was significantly higher than that of genotype 2 (78.0 %, $p = 0.015$). For genotype 1, the SVR rate of IL28B genotype TT was significantly higher ($p = 0.016$) than that of genotype TG or GG. The SVR rates in group L and group S were not significantly different for IL28B genotypes TT, TG, or GG. The SVR rate of genotype TT was significantly higher than that of genotype TG or GG. The SVR rates in groups L and S were evaluated for the CAP/CTM HCV RNA values and ART before therapy, but no significant difference was

Table 3 HCV RNA concentrations for HCV genotypes 1 and 2 during PEG-IFN/RBV treatment as measured by two real-time RT-PCR assays: CAP/CTM and ART

Genotype	*n*	CAP/CTM (log IU/ml)		ART (log IU/ml)		Average HCV RNA level (log IU/ml)		Quantitation difference (CAP/CTM−ART)		*p* value*
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Genotype 1										
Before treatment	55	6.06	1.09	5.52	1.09	5.00	1.09	0.54	0.21	0.0161
Day 1	53	4.64	1.11	4.23	1.12	4.44	1.12	0.41	0.15	0.0662
Week 1	55	3.79	1.93	3.41	1.74	3.60	1.83	0.38	0.38	0.2739
Week 2	55	3.06	2.05	2.80	1.74	2.93	1.88	0.26	0.49	0.4966
Genotype 2										
Before treatment	42	5.94	1.21	5.65	1.15	5.79	1.17	0.28	0.34	0.2734
Day 1	39	4.55	1.19	4.49	1.08	4.52	1.13	0.06	0.27	0.8618
Week 1	41	3.39	1.51	3.21	1.48	3.30	1.47	0.18	0.45	0.6946
Week 2	42	1.98	1.30	1.89	1.06	1.96	1.14	0.09	0.29	0.8014

CAP/CTM Cobas Ampliprep/Cobas TaqMan, *ART* Abbott real time HCV test

* CAP/CTM versus ART

observed. The SVR rate of patients with less than 6.0 log IU/ml of the HCV RNA values measured by both CAP/CTM and assay was higher than that with 6.0 log IU/ml or more, but this difference was not significant. For genotype 2, the SVR rate of IL28B genotype TT was higher than that of genotype TG or GG, but this difference also was not significant. There was no difference in the SVR rates between less than 6.0 and 6.0 log IU/ml or more. The SVR rates in group L and group S were not significantly different. The SVR rate of patients with genotype 2 was high regardless of HCV RNA values and IL28B genotype.

Discussion

The study revealed that the HCV RNA values measured by CAP/CTM were higher than those by ART among the subjects with genotype 1; however, no difference was observed among the patients with genotype 2. The difference of HCV RNA values (CAP/CTM minus ART) in genotype 1 was significantly higher than those in genotype 2 ($p < 0.0001$). The positive rate (>0) of the difference of HCV RNA values (CAP/CTM minus ART) in genotype 1 was 100 %, which was significantly higher than the positive rate of 78.6 % in the genotype 2 group ($p < 0.001$). The differences of HCV RNA values (CAP/CTM minus ART) in the genotype 1 group were all positive (>0), and all the viral load measurements obtained from CAP/CTM were higher than those from ART (Fig. 3). Ohnishi et al. [11] reported that the difference of the WHO standard versions used for each assay calibration might be the reason for the findings. CAP/CTM adopted the First International HCV RNA WHO standard for the assay calibration, while ART adopted the Second International HCV RNA WHO standard. Direct comparison of the two assays for measuring the WHO standard revealed a consistently higher quantitation of the WHO standard by CAP/CTM than by ART. The HCV RNA values of CAT/CTM in genotype 2 were reported to be lower those of ART [14]. Some cases in this evaluation also had lower CAP/CTM HCV RNA values (Fig. 3). No consistency was observed in genotype 2; some CAT/CTM HCV RNA values were higher than ART and some were lower. Base substitution is thought to contribute to this inconsistency [14]; this could have resulted from the differences between the two PCR methods. Also, this is consistent with a previous study in which CAP/CTM values were relatively higher for genotype 1 and lower for genotype 2 [2].

The difference of the HCV RNA values between CAP/CTM and ART was investigated in this study based on viral kinetics from the early stage of PEG-IFN/RBV treatment. After administration of PEG-IFN/RBV treatment, reduction of HCV RNA measurements obtained from both the CAP/CTM assay and the ART assay was observed from day 1 regardless of genotype (1 or 2). The HCV RNA values of CAP/CTM were consistently higher than those of ART during PEG-IFN/RBV therapy. The difference between these two values eventually became smaller because of the effect of PEG-IFN/RBV therapy, and a significant difference was no longer observed.

The IL28B genotype is one of the predictors of PEG-IFN/RBV therapy outcome before administration of treatment [15, 16]. In this study, for the genotype 1 patients, the SVR rate of IL28B genotype TT was significantly higher than the SVR rates of genotype TG or GG.

Table 4 Sustained virological response (SVR) rate in patients who received PEG-IFN/RBV therapy by difference between CAT/CTM HCV value and ART value before PEG-IFN/RBV therapy

	Group S SVR/*n* (%)	Group L SVR/*n* (%)	*p* value*	Total SVR/*n* (%)
Genotype 1				
IL28B				
TT	15/19 (79)	10/19 (53)	0.087	25/38 (66)
TG or GG	1/5 (20)	2/9 (22)	0.481	3/14 (21)
p value	0.012	0.128		0.004
CAP/CTM				
<6.0 log IU/ml	6/8 (75)	5/7 (71)	0.875	11/15 (73)
≥6.0 log IU/ml	10/16 (63)	7/21 (33)	0.077	17/37 (46)
p value	0.540	0.077		0.072
ART				
<6.0 log IU/ml	8/10 (80)	8/14 (57)	0.241	16/24 (67)
≥6.0 log IU/ml	8/14 (57)	4/14 (29)	0.126	12/28 (43)
p value	0.241	0.126		0.085
Total	16/24 (67)	12/28 (43)	0.086	28/52 (54)†
Genotype 2				
IL28B				
TT	19/22 (86)	4/6 (67)	0.264	23/28 (82)
TG or GG	5/7 (71)	4/6 (67)	0.852	9/13 (69)
p value	0.362	1.000		0.112
CAP/CTM				
<6.0 log IU/ml	11/15 (73)	3/4 (75)	0.946	14/19 (74)
≥6.0 log IU/ml	12/14 (86)	6/8 (75)	0.531	18/22 (82)
p value	0.411	1.000		0.530
ART				
<6.0 log IU/ml	14/15 (93)	4/5 (80)	0.717	18/20 (90)
≥6.0 log IU/ml	10/14 (71)	4/7 (57)	0.305	14/21 (67)
p value	0.564	0.407		0.293
Total	24/29 (83)	8/12 (67)	0.257	32/41 (78)†

Group L, ≥0.5 log IU/ml (CAP/CTM−ART); group S, <0.5 log IU/ml (CAP/CTM−ART)

* Group S versus group L

† $p = 0.015$, genotype 1 versus genotype 2

The HCV RNA values obtained from the two real-time PCR assays were analyzed based on the IL28B genotypes in this study. The HCV RNA values in the TT genotype group measured by CAP/CTM were significantly higher than those by ART; however, there was no significant difference in the TG or GG genotype groups. IL28B genotypes TT, TG, or GG were evaluated by differences of HCV RNA values between the CAP/CTM and ART: only the genotype TT group had a higher SVR rate. No SVR rate difference depending on the difference of HCV RNA values between CAP/CTM and ART was observed for genotypes 1 and 2. Clinically, a higher SVR rate was observed in the genotype TT group. It is assumed that the HCV RNA values of CAP/CTM were significantly higher than those of ART in genotype 1 patients because 73 % were in the genotype TT group. Therefore, there is assumed to be no correlation between IL28B and the difference of HCV RNA values between CAP/CTM and ART.

The data were also analyzed based on gender, age, and the number of platelets. For all variables, HCV RNA values as measured by CAP/CTM were significantly higher than those by ART; however, there was no difference in the HCV RNA values measured by CAP/CTM and ART when the measurements were compared against each variable.

The difference in HCV RNA measurements is suggested to be the result of HCV genotype. The prevalence of genotype 1 is higher in Japanese; therefore, the difference was observed in the measurements obtained from both assays.

The details of primer design and the PCR protocol for the products of both manufacturers used for this evaluation

are not disclosed. The PCR protocol of CAP/CTM method has two steps whereas the ART method has a three-step protocol. For the CAP/CTM method, elongation and probe hybridization are conducted simultaneously in the low-temperature step, and the temperature is generally 50–60 °C . In the ART method, a single-stranded linear probe is used instead of a TaqMan Probe and it has three steps, although it is also a real-time PCR method. Also, probe hybridization takes place at a lower temperature than for the CAP/CTM method, which is thought to optimize the tolerance level for HCV detection.

The newly developed ART features nucleic acid extraction using m2000 system, automated real-time PCR analysis, and high processing capacity. The assay results correlate well with the CAP/CTM assay, which suggests the wide application of the platform in clinical settings in the future. Additionally, the sample volume is 0.5 or 0.2 ml, which is highly practical for pediatric patients or when only a limited amount of patient sample is available. Also, some research has suggested that the genotype reactivity of ART is superior [17, 18].

In this study, the SVR rate was higher in genotype 2 than in genotype 1. For genotype 1, the SVR rate in IL28B genotype TT was higher than that in genotype TG or GG. For genotype 2, there was no difference of SVR rate between genotype TT and genotype TG or GG. These results were similar to the results of a previous study.

In summary, the HCV RNA values in genotype 1 obtained from the CAP/CTM assay were significantly higher compared to the values obtained from ART; however, no difference was observed in genotype 2. The HCV RNA values decreased during PEG-IFN/RBV therapy regardless of genotype. The HCV RNA value for CAP/CTM during PEG-IFN/RBV therapy was consistently higher than that for ART. However, the difference in these two values gradually became less during the course of therapy, and eventually no significant difference was observed near the detection level. No correlation was observed between the SVR rate and the difference between the CAP/CTM HCV values and the ART HCV value before treatment. Both CAP/CTM assay and ART assay were useful for PEG-IFN/RBV therapy. In this study, it was not clear which of the two HCV RNA assays was useful regarding the effects of IFN therapy. More detailed study is necessary.

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Original Article

Factors responsible for the discrepancy between *IL28B* polymorphism prediction and the viral response to peginterferon plus ribavirin therapy in Japanese chronic hepatitis C patients

Hiroaki Saito,[1,2] Kiyoaki Ito,[1] Masaya Sugiyama,[1] Teppei Matsui,[1] Yoshihiko Aoki,[1] Masatoshi Imamura,[1] Kazumoto Murata,[1] Naohiko Masaki,[1] Hideyuki Nomura,[3] Hiroshi Adachi,[4] Shuhei Hige,[5] Nobuyuki Enomoto,[6] Naoya Sakamoto,[7] Masayuki Kurosaki,[8] Masashi Mizokami[1] and Sumio Watanabe[2]

[1]*The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa,* [2]*Department of Gastroenterology, Juntendo University School of Medicine, Bunkyoku,* [3]*The Center for Liver Diseases, Shin-Kokura Hospital, Kitakyushu, Fukuoka,* [4]*Department of Virology and Liver Unit, Tonami General Hospital, Tonami,* [5]*Department of Internal Medicine, Hokkaido University Graduate School of Medicine, Sapporo,* [6]*Department of Internal Medicine, University of Yamanashi, Kofu,* [7]*Department for Hepatitis Control, Tokyo Medical and Dental University, Tokyo, and* [8]*Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Musashino, Japan*

Aim: *IL28B* polymorphisms serve to predict response to pegylated interferon plus ribavirin therapy (PEG IFN/RBV) in Japanese patients with chronic hepatitis C (CHC) very reliably. However, the prediction by the *IL28B* polymorphism contradicted the virological response to PEG IFN/RBV in some patients. Here, we aimed to investigate the factors responsible for the discrepancy between the *IL28B* polymorphism prediction and virological responses.

Methods: CHC patients with genotype 1b and high viral load were enrolled in this study. In a case–control study, clinical and virological factors were analyzed for 130 patients with rs8099917 TT genotype and 96 patients with rs8099917 TG or GG genotype who were matched according to sex, age, hemoglobin level and platelet count.

Results: Higher low-density lipoprotein (LDL) cholesterol, lower γ-glutamyltransferase and the percentage of wild-type phenotype at amino acids 70 and 91 were significantly associated with the rs8099917 TT genotype. Multivariate analysis showed that rs8099917 TG or GG genotype, older age and lower LDL cholesterol were independently associated with the non-virological responder (NVR) phenotype. In patients with rs8099917 TT genotype (predicted as virological responder [VR]), multivariate analysis showed that older age was independently associated with NVR. In patients with rs8099917 TG or GG genotype (predicted as NVR), multivariate analysis showed that younger age was independently associated with VR.

Conclusion: Patient age gave rise to the discrepancy between the prediction by *IL28B* polymorphism and the virological responses, suggesting that patients should be treated at a younger age.

Key words: aging, genotype, IL28B, low-density lipoprotein cholesterol, single nucleotide polymorphism

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is a global health problem with worldwide estimates of 120–130 million carriers.[1] Chronic HCV infection, the leading cause of liver transplantation, can lead to progressive liver disease, resulting in cirrhosis and complications, including decompensated liver disease and hepatocellular carcinoma.[2] The current standard-of-care treatment for suitable patients with chronic HCV infection consists of pegylated interferon-α-2a or -2b (PEG IFN) given by injection in combination with oral ribavirin (RBV) for 24 or 48 weeks, depending on HCV genotype. Large-scale treatment in the USA and Europe showed that 42–52% of patients with HCV genotype 1

Correspondence: Dr Masashi Mizokami, The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, 1-7-1 Kohnodai, Ichikawa, Chiba 272-8516, Japan. Email: mmizokami@hospk.ncgm.go.jp

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achieved a sustained virological response (SVR),[3–5] and studies conducted in Japan produced similar results. This treatment is associated with well-known side-effects (e.g. influenza-like syndrome, hematological abnormalities and neuropsychiatric events) resulting in reduced compliance and fewer patients completing treatment.[6] It is important to predict an individual's response before treatment with PEG IFN/RBV to avoid side-effects, as well as to reduce the treatment cost. The HCV genotype, in particular, is used to predict the response: patients with the HCV genotype 2/3 have a relatively high rate of SVR (70–80%) with 24 weeks of treatment, whereas those infected with genotype 1 have a much lower rate of SVR, despite 48 weeks of treatment.[5]

Our recent genome-wide association studies (GWAS) revealed that several highly correlated common single nucleotide polymorphisms (SNP) in the region of the interleukin-28B (*IL28B*) gene on chromosome 19, coding for interferon (IFN)-λ3, are implicated in the non-virological responder (NVR) to PEG IFN/RBV phenotype among patients infected by HCV genotype 1.[7] The association between response to PEG IFN/RBV and SNP associated with *IL28B* was concurrently reported by two other groups who also employed GWAS.[8,9] The *IL28B* polymorphism was highly predictive of the response to PEG IFN/RBV therapy in Japanese chronic hepatitis C (CHC) patients.[10–12] However, this was not always the case. Therefore, we attempted to determine why the *IL28B* polymorphism did not predict the response of all patients. The nature of the functional link between the *IL28B* polymorphism and HCV clearance is unknown, and this must be defined to understand how the *IL28B* polymorphism correlates with HCV clearance. Therefore, we also investigated the association between the *IL28B* polymorphism and clinical characteristics of CHC patients.

METHODS

Patients

A TOTAL OF 696 CHC patients with genotype 1b and high viral load were recruited from the National Center for Global Health and Medicine, Hokkaido University Hospital, Tokyo Medical and Dental University Hospital, Yamanashi University Hospital, Tonami General Hospital, and Shin-Kokura Hospital in Japan. In a case–control study, sex, age, hemoglobin level and platelet count were matched between patients with the rs8099917 TT genotype ($n = 130$) and patients with rs8099917 TG or GG genotypes ($n = 96$) to eliminate background biases.

Each patient was treated with PEG IFN-α-2b (1.5 μg/kg s.c. weekly) or PEG IFN-α-2a (180 μg/body s.c. weekly) plus RBV (600–1000 mg daily, depending on bodyweight). Because a reduction in the dose of PEG IFN/RBV can contribute to a lower SVR rate,[13] only patients with an adherence of more than 80% dose for both drugs during the first 12 weeks were included in this study. Those positive for hepatitis B surface antigen and/or anti-HIV were excluded from this study.

Non-virological response was defined as less than a 2 log-unit decline in the serum level of HCV RNA from the pretreatment baseline value within the first 12 weeks and detectable viremia 24 weeks after treatment. Virological response (VR) was defined as attaining SVR or transient virological response (TVR) in this study; SVR was defined as undetectable HCV RNA in serum 6 months after treatment, whereas TVR was defined as a reappearance of HCV RNA in serum after the treatment was discontinued for a patient who had undetectable HCV RNA during the therapy or on completion of the therapy. At the time of enrollment, written informed consent was obtained for the collection and storage of serum and peripheral blood. This study was conducted in accordance with provisions of the Declaration of Helsinki.

Clinical and laboratory data

The sex, age, hemoglobin (Hb) and platelet counts were matched between study groups. Other parameters determined were as follows: alkaline phosphatase (ALP), alanine transaminase (ALT), total cholesterol, fasting blood sugar (FBS), low-density lipoprotein (LDL) cholesterol, γ-glutamyl transpeptidase (γ-GTP), α-fetoprotein (AFP), HCV RNA level and the rs8099917 polymorphism near *IL28B*.

DNA extraction

Genomic DNA was extracted from the buffy coat fraction of patients' whole blood using a GENOMIX kit (Talent SRL; Trieste, Italy).

IL28B genotyping

We have reported that the rs8099917 polymorphism is the best predictor for the response of Japanese CHC patients to PEG IFN/RBV therapy than other SNP near *IL28B*.[14] Therefore, the rs8099917 polymorphism was genotyped using the InvaderPlus assay (Third Wave Japan, Tokyo, Japan), which combines polymerase

chain reaction (PCR) and the invader reaction.[15,16] The InvaderPlus assay was performed using the LightCycler LC480 (Roche Applied Science, Mannheim, Germany).

Detection of amino acid substitutions in core and NS5A regions of HCV-1b

In the present study, substitutions of amino acid residues 70 (s-aa 70) and 91 (s-aa 91), and the presence of the IFN sensitivity-determining region (ISDR) were determined by direct nucleotide sequencing. HCV RNA was extracted from serum samples at the start of patients' therapy and reverse transcribed with a random primer and SuperScript III reverse transcriptase (Life Technologies, Carlsbad, CA, USA). Nucleic acids were amplified by PCR as described.[17]

Statistical analysis

Quantitative variables were expressed as the mean ± standard error (SE) unless otherwise specified. Categorical variables were compared using a χ^2-test or Fisher's exact test, as appropriate, and continuous variables were compared using the Mann–Whitney *U*-test. $P < 0.05$ was considered statistically significant. Multivariate analysis was performed using a stepwise logistic regression model. We performed statistical analyses using STATA ver. 11.0 (StataCorp, College Station, TX, USA).

RESULTS

Patient characteristics and *IL28B* genotype in a matched case–control study

TABLE 1 SHOWS PATIENT characteristics according to *IL28B* genotype. In a matched case–control study, sex, age, Hb levels and platelet counts were matched between 130 patients with rs8099917 TT genotype and 96 patients with rs8099917 TG or GG genotype. Lower γ-GTP ($P = 0.013$) and higher LDL cholesterol levels ($P < 0.001$) were significantly associated with the TT genotype of rs8099917. The percentages of wild type of s-aa 70 and s-aa 91 of patients with the rs8099917 TT genotype were significantly higher than those of patients with rs8099917 TG or GG genotype (s-aa 70: TT vs TG + GG, 68% vs 37% [$P < 0.001$]; s-aa 91: TT vs TG + GG, 68% vs 51% [$P = 0.017$]).

Factors associated with NVR in total patients

Table 2 shows the factors associated with NVR by univariate and multivariate analyses. Univariate analysis showed that older age ($P = 0.002$), lower platelet counts ($P = 0.01$), higher γ-GTP ($P = 0.013$), lower total cholesterol ($P = 0.017$), lower LDL cholesterol ($P < 0.001$) levels and higher AFP levels ($P = 0.019$) were significantly associated with NVR. The percentage of TG or GG genotype of rs8099917 of patients with NVR was

Table 1 Univariate analysis of *IL28B* TT and TG + GG genotypes

Variable	TT genotype ($n = 130$)	TG + GG genotype ($n = 96$)	*P*-value
Sex (% male)	61 (47)	46 (48)	Matched
Age (years), mean (SE)	57.2 (0.8)	57.5 (0.9)	Matched
Hemoglobin (g/dL), mean (SE)	14.3 (0.3)	13.9 (0.2)	Matched
Platelet count (/μL), mean (SE)	16.2 (0.5)	16.0 (0.5)	Matched
ALT (IU/L), mean (SE)	79.4 (5.4)	80.5 (7.8)	0.281
ALP (IU/L), mean (SE)	273.8 (11.7)	283.9 (11.8)	0.313
γ-GTP (IU/L), mean (SE)	63.4 (6.0)	76.0 (6.4)	0.013
Total cholesterol (mg/dL), mean (SE)	177.5 (3.3)	172.3 (3.2)	0.345
LDL cholesterol (mg/dL), mean (SE)	99.0 (2.6)	83.5 (2.8)	<0.001
Fasting blood sugar (mg/dL), mean (SE)	114.1 (4.1)	104.4 (1.9)	0.97
AFP (ng/dL), mean (SE)	9.8 (1.1)	11.5 (1.6)	0.190
HCV RNA (log IU), mean (SE)	6.2 (0.1)	6.1 (0.1)	0.186
s-aa 70 wild type (%)	70/103 (68)	30/81 (37)	<0.001
s-aa 91 wild type (%)	70/103 (68)	41/81 (51)	0.017
ISDR mutation 0–1 point (%)	82/100 (82)	70/81 (86)	0.42

AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; LDL, low-density lipoprotein; SE, standard error.

Table 2 Univariate and multivariate analyses of patients with chronic hepatitis C treated with PEG IFN/RBV with respect to VR and NVR

Variable	Univariate analysis			Multivariate analysis	
	VR ($n = 128$)	NVR ($n = 98$)	*P*-value	OR (95% CI)	*P*-value
Sex (% male)	65 (51)	42 (43)	0.237		
Age (years), mean (SE)	55.6 (0.8)	59.6 (0.9)	0.002	1.075 (1.012–1.143)	0.02
rs8099917 (TG or GG genotype) (%)	23/128 (18)	73/98 (74)	<0.001	25.460 (7.436–87.169)	<0.001
Hemoglobin (g/dL), mean (SE)	14.4 (0.3)	13.7 (0.2)	0.053		
Platelet count (/μL), mean (SE)	16.9 (0.5)	15.0 (0.5)	0.01		
ALT (IU/L), mean (SE)	83.9 (6.4)	74.5 (6.2)	0.116		
ALP (IU/L), mean (SE)	274.1 (12.3)	282.9 (11.2)	0.169		
γ-GTP (IU/L), mean (SE)	65.9 (6.4)	72.6 (5.6)	0.013		
Total cholesterol (mg/dL), mean (SE)	180.3 (3.1)	168.4 (3.5)	0.017		
LDL cholesterol (mg/dL), mean (SE)	100.5 (2.7)	83.5 (2.8)	<0.001	0.978 (0.956–0.999)	0.046
Fasting blood sugar (mg/dL), mean (SE)	106.6 (2.9)	114.8 (4.4)	0.058		
AFP (ng/dL), mean (SE)	9.6 (1.1)	12.0 (1.6)	0.021		
HCV RNA (Log IU), mean (SE)	6.2 (0.1)	6.2 (0.1)	0.876		
s-aa 70 wild type (%)	67/102 (66)	33/82 (54)	0.001		
s-aa 91 wild type (%)	67/102 (66)	44/82 (54)	0.097		
ISDR mutation 0–1 point (%)	79/96 (82)	73/85 (86)	0.511		

AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; LDL, low-density lipoprotein; NVR, non-virological response; OR, odds ratio; PEG IFN, peginterferon; SE, standard error; RBV, ribavirin; VR, virological response.

significantly higher than that of patients with VR (VR vs NVR: 23/128 [18%] vs 73/98 [74%], $P < 0.001$). The percentage of wild-type s-aa 70 in patients with NVR was significantly lower than that in patients with VR [VR vs NVR: 67/102 [66%] vs 33/82 [54%], $P = 0.001$]. Multivariate analysis showed that older age (odds ratio [OR] = 1.075; 95% confidence interval [CI] = 1.012–1.14; $P = 0.02$), TG or GG genotype of rs8099917 (OR = 25.460; 95% CI = 7.436–87.169; $P < 0.001$) and lower LDL cholesterol levels (OR = 0.978; 95% CI = 0.956–0.999; $P = 0.046$) were independently associated with NVR.

VR to treatment depending on *IL28B* genotype

In the patients with the rs8099917 TT genotype, the rates of SVR, TVR and NVR were 62%, 19% and 19%, respectively. Therefore, 19% patients were NVR, even though rs8099917 represents the TT genotype (predicted as VR). In contrast, in the patients with rs8099917 TG or GG, the rates of SVR, TVR and NVR were 14%, 10% and 76%, respectively. Therefore, 24% patients were VR, even though rs8099917 was TG or GG genotype (predicted as NVR) (Fig. 1).

Factors associated with NVR in patients with the rs8099917 TT genotype

Table 3 shows the factors associated with NVR in patients with the rs8099917 TT genotype (predicted as VR) by univariate and multivariate analyses. Univariate analysis showed that female sex ($P = 0.003$), older age

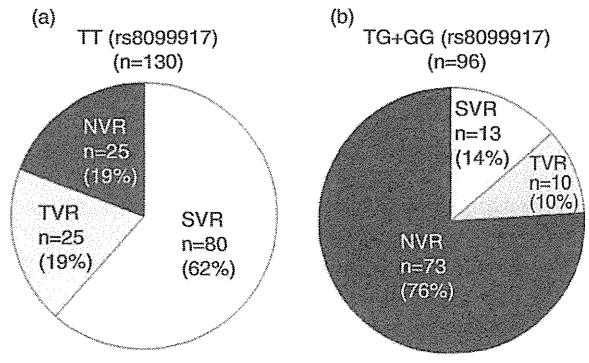

Figure 1 Virological responses to pegylated interferon and ribavirin therapy were shown in patients with rs8099917 TT (a) and TG + GG (b). NVR, non-virological response; SVR, sustained virological response; TVR, transient virological response.

Table 3 Variables associated with NVR by univariate and multivariate analyses in patients with rs8099917 TT genotype

Variable	Univariate analysis			Multivariate analysis	
	VR ($n = 105$)	NVR ($n = 25$)	*P*-value	OR (95% CI)	*P*-value
Sex (% male)	56 (53)	5 (20)	0.003		
Age (years), mean (SE)	56.1 (0.8)	61.7 (1.6)	0.001	1.142 (1.026–1.271)	0.015
Hemoglobin (g/dL), mean (SE)	14.6 (0.4)	13.1 (0.3)	0.005		
Platelet count (/μL), mean (SE)	16.7 (0.6)	13.8 (1.0)	0.019		
ALT (IU/L), mean (SE)	83.6 (6.3)	61.0 (7.9)	0.053		
ALP (IU/L), mean (SE)	270.6 (13.6)	285.9 (22.3)	0.206		
γ-GTP (IU/L), mean (SE)	66.9 (7.1)	49.2 (7.4)	0.473		
Total cholesterol (mg/dL), mean (SE)	180.2 (3.6)	165.0 (7.6)	0.072		
LDL cholesterol (mg/dL), mean (SE)	101.2 (2.9)	88.5 (5.2)	0.067		
Fasting blood sugar (mg/dL), mean (SE)	108.4 (3.5)	140.0 (15.5)	0.127		
AFP (ng/dL), mean (SE)	9.4 (1.2)	12.2 (3.6)	0.245		
HCV RNA (log IU), mean (SE)	6.2 (0.1)	6.2 (0.1)	0.948		
s-aa 70 wild type (%)	57/83 (66)	13/20 (75)	0.752		
s-aa 91 wild type (%)	55/83 (66)	15/20 (75)	0.452		
ISDR mutation 0–1 point (%)	64/79 (81)	18/21 (86)	0.618		

AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; LDL, low-density lipoprotein; NVR, non-virological response; OR, odds ratio; SE, standard error; VR, virological response.

($P = 0.001$), lower Hb levels ($P = 0.005$) and lower platelet counts ($P = 0.019$) were significantly associated with NVR in patients with the rs8099917 TT genotype. Multivariate analysis showed that only older age was independently associated with NVR in patients with the rs8099917 TT genotype (predicted as VR) (OR = 1.142; 95% CI = 1.026–1.27; $P = 0.015$).

Factors associated with VR in patients with the rs8099917 TG or GG genotypes

Table 4 shows the factors associated with VR in patients with the rs8099917 TG or GG genotypes (predicted as NVR) by univariate and multivariate analyses. Younger age ($P = 0.005$), lower γ-GTP ($P = 0.009$) and higher LDL cholesterol levels ($P = 0.032$) were significantly associated with VR by univariate analysis. Multivariate analysis showed that only younger age was independently associated with VR in patients with the rs8099917 TG or GG genotype (predicted as NVR) (OR = 0.926; 95% CI = 0.867–0.990; $P = 0.023$).

Rate of VR depending on the rs8099917 genotype of each age group

We divided patients into four age groups and compared VR rates by the differences in rs8099917 genotype for each group. The rate of VR decreased gradually in the older age groups independent of genotype. In the less than 49 years age group, the rate of VR in patients with the rs8099917 TT genotype was significantly higher than that in patients with the rs8099917 TG + GG genotypes ($P = 0.0002$). Further, in the 50–59 and 60–69 years age groups, the rates of VR in patients with the rs8099917 TT genotype were significantly higher than those in patients with the rs8099917 TG + GG genotypes ($P < 0.0001$, respectively). In the group that included subjects aged older than 69 years, only 50% of patients achieved VR even in those with the rs8099917 TT genotype (predicted as VR). In contrast, 47.6% of patients achieved VR, including those with the rs8099917 TG or GG genotypes (predicted as NVR) in the less than 49 years group (Fig. 2).

DISCUSSION

SINGLE NUCLEOTIDE POLYMORPHISM array analysis employing GWAS technology conducted by our laboratory and others revealed the relationships between SNP associated with the *IL28B* locus or present within the coding sequences for IFN-λ3, or the response to PEG IFN/RBV therapy for CHC.[7–9] Subsequent studies have confirmed that the response to PEG IFN/RBV therapy correlates with the SNP associated with *IL28B*[18,19] and indicates their value for predicting the response to PEG IFN/RBV therapy. Unfortunately, these predictions do not hold for some patients. In an attempt to understand the reasons for this, in the present study,

Table 4 Variables associated with VR by univariate and multivariate analyses in patients with rs8099917 TG or GG genotypes

Variable	Univariate analysis			Multivariate analysis	
	VR ($n = 23$)	NVR ($n = 73$)	*P*-value	OR (95% CI)	*P*-value
Sex (% male)	9 (40%)	37 (51%)	0.333		
Age (years), mean (SE)	53.2 (1.7)	58.8 (1.1)	0.005	0.926 (0.867–0.990)	0.023
Hemoglobin (g/dL), mean (SE)	13.6 (0.3)	13.9 (0.2)	0.44		
Platelet count (/μL), mean (SE)	17.6 (1.1)	15.5 (0.6)	0.059		
ALT (IU/L), mean (SE)	85.5 (21.6)	78.9 (7.8)	0.767		
ALP (IU/L), mean (SE)	291.9 (28.6)	281.8 (13.0)	0.921		
γ-GTP (IU/L), mean (SE)	62.2 (15.1)	80.4 (6.9)	0.009		
Total cholesterol (mg/dL), mean (SE)	180.5 (6.2)	169.5 (3.7)	0.17		
LDL cholesterol (mg/dL), mean (SE)	97.6 (6.9)	81.9 (3.6)	0.032		
Fasting blood sugar (mg/dL), mean (SE)	98.1 (2.8)	106.3 (2.3)	0.084		
AFP (ng/dL), mean (SE)	10.3 (3.4)	11.9 (1.8)	0.123		
HCV RNA (log IU), mean (SE)	5.9 (0.1)	6.2 (0.1)	0.087		
s-aa 70 wild type (%)	10/19 (53)	20/62 (32)	0.108		
s-aa 91 wild type (%)	12/19 (63)	29/62 (47)	0.211		
ISDR mutation 0–1 point (%)	15/17 (88)	55/64 (86)	0.806		

AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; LDL, low-density lipoprotein; NVR, non-virological response; OR, odds ratio; SE, standard error; VR, virological response.

we recruited a new set of patients for further analysis. Here, we confirmed that *IL28B* polymorphism was the most significant predictive factor for NVR with respect to PEG IFN/RBV treatment. Moreover, 19% of patients exhibiting the rs8099917 TT genotype were NVR, although they were predicted as VR. Twenty-four percent of patients with the rs8099917 TG or GG genotypes were VR, although they were predicted as NVR. We were able to determine by multivariate analysis that age was the most likely factor responsible for the discordance

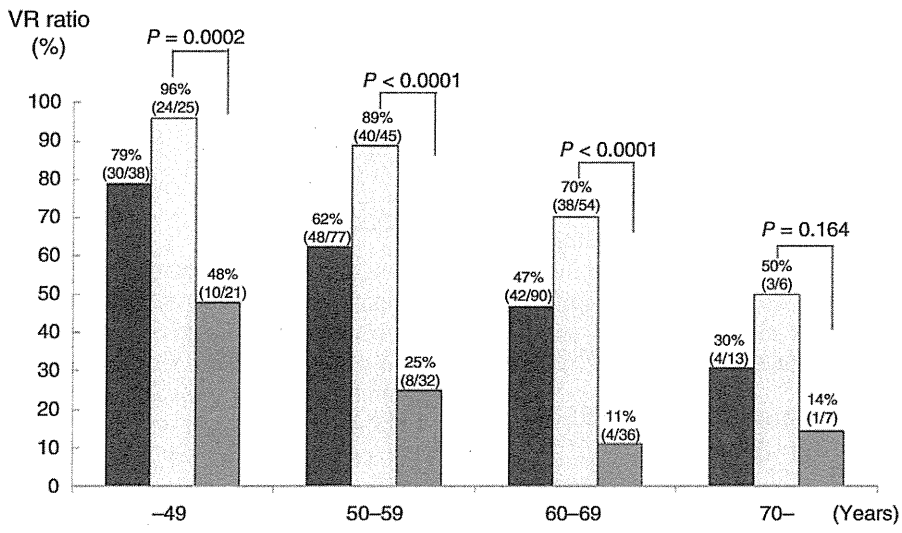

Figure 2 Virological responses (VR) to pegylated interferon and ribavirin therapy were compared between the patients with rs8099917 TT and TG + GG in each generation group. (■) Total patients, (□) TT genotype (rs8099917), (▣) TG + GG genotype (rs8099917).

between *IL28B* genotype and patients' response to viral infection.

How does age influence the VR to PEG IFN/RBV therapy? First, the lower rate of VR to PEG IFN/RBV therapy in patients with CHC was attributed to lower compliance with the IFN or RBV dose.[20,21] Because lower compliance with PEG IFN or RBV therapy was expected to be associated with a lower rate of VR in older patients, we recruited patients who were administrated over 80% of the prescribed dose of IFN/RBV. Therefore, lower compliance can be discounted as a reason for reduced response. Second, a more advanced stage of fibrosis might have been present in the older group. Platelet counts in patients with NVR were significantly lower than those in patients with VR, and lower platelet counts may be associated with advanced fibrosis.[22] Moreover, advanced fibrosis is associated with lower rates of SVR to IFN-based therapy.[23] Third, epigenetic factors such as DNA methylation induced by aging may be involved in the reduced efficacy of PEG IFN/RBV treatment in older patients. DNA methylation near gene promoters is known to turn off transcription or reduce it considerably,[24] and advanced age is strongly associated with the increased DNA methylation.[25] Therefore, DNA methylation may be increased near or in the *IL28B* promoter as a function of age resulting in suppression of *IL28B* transcription.

Lower LDL cholesterol levels were significantly associated with NVR in patients with CHC. Moreover, LDL cholesterol levels in patients with the rs8099917 TT genotype were significantly higher than those in patients with the TG + GG genotypes. The association between LDL cholesterol and *IL28B* polymorphism as well as the VR to PEG IFN/RBV has been reported.[26] Higher pretreatment levels of LDL cholesterol have been shown to predict increased response to standard PEG IFN/RBV treatment for patients with CHC.[27,28] Although the mechanisms responsible for the association between LDL cholesterol levels and the VR to PEG IFN/RBV are unknown, the *IL28B*-rs8099917 TT responder genotype, which may correlate with an increased likelihood of treatment response and higher LDL cholesterol levels, is associated with either lower IFN-λ3 activity or reduced expression of genes regulated by IFN-mediated signaling pathways.

In conclusion, our studies provide compelling evidence that patient age is most likely responsible for incorrect predictions of VR to PEG IFN/RBV therapy in Japanese CHC patients based on *IL28B* genotypes. Our findings indicated that patients should be treated as soon as they are diagnosed. It will be important to investigate the role of the epigenetic factors associated with *IL28B* expression to develop more effective PEG IFN/RBV-based therapies for patients with CHC.

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LecT-Hepa, a Glyco-Marker Derived from Multiple Lectins, as a Predictor of Liver Fibrosis in Chronic Hepatitis C Patients

Kiyoaki Ito,[1] Atsushi Kuno,[2] Yuzuru Ikehara,[2] Masaya Sugiyama,[1] Hiroaki Saito,[1] Yoshihiko Aoki,[1] Teppei Matsui,[1] Masatoshi Imamura,[1] Masaaki Korenaga,[1] Kazumoto Murata,[1] Naohiko Masaki,[1] Yasuhito Tanaka,[3] Shuhei Hige,[4] Namiki Izumi,[5] Masayuki Kurosaki,[5] Shuhei Nishiguchi,[6] Michiie Sakamoto,[7] Masayoshi Kage,[8] Hisashi Narimatsu,[2] and Masashi Mizokami[1]

Assessment of liver fibrosis in patients with chronic hepatitis C (CHC) is critical for predicting disease progression and determining future antiviral therapy. LecT-Hepa, a new glyco-marker derived from fibrosis-related glyco-alteration of serum alpha 1-acid glycoprotein, was used to differentiate cirrhosis from chronic hepatitis in a single-center study. Herein, we aimed to validate this new glyco-marker for estimating liver fibrosis in a multicenter study. Overall, 183 CHC patients were recruited from 5 liver centers. The parameters *Aspergillus oryzae* lectin (AOL) / *Datura stramonium* lectin (DSA) and *Maackia amurensis* lectin (MAL)/DSA were measured using a bedside clinical chemistry analyzer in order to calculate LecT-Hepa levels. The data were compared with those of seven other noninvasive biochemical markers and tests (hyaluronic acid, tissue inhibitor of metalloproteases-1, platelet count, aspartate aminotransferase-to-platelet ratio index [APRI], Forns index, Fib-4 index, and Zeng's score) for assessing liver fibrosis using the receiver-operating characteristic curve. LecT-Hepa correlated well with the fibrosis stage as determined by liver biopsy. The area under the curve (AUC), sensitivity, and specificity of LecT-Hepa were 0.802, 59.6%, and 89.9%, respectively, for significant fibrosis; 0.882, 83.3%, and 80.0%, respectively, for severe fibrosis; and 0.929, 84.6%, and 88.5%, respectively, for cirrhosis. AUC scores of LecT-Hepa at each fibrosis stage were greater than those of the seven aforementioned noninvasive tests and markers. *Conclusion*: The efficacy of LecT-Hepa, a glyco-marker developed using glycoproteomics, for estimating liver fibrosis was demonstrated in a multicenter study. LecT-Hepa given by a combination of the two glyco-parameters is a reliable method for determining the fibrosis stage and is a potential substitute for liver biopsy. (HEPATOLOGY 2012;56:1448-1456)

Accurate staging of hepatic fibrosis in patients with chronic hepatitis C (CHC) is most important for predicting disease progression and determining the need for initiating antiviral therapy, such as interferon (IFN) therapy.[1,2] Liver biopsy has been considered the gold standard for fibrosis staging for many years.[3] However, liver biopsy is invasive and painful,[4,5] with rare but potentially life-threatening complications.[6] In addition, this method may suffer from sampling errors since only 1/50,000 of the organ is examined.[7] Furthermore, inter- and intraobserver discrepancies reaching levels of 10% to 20% have been

Abbreviations: α2-MG, α2-macroglobulin; AFP, alpha-fetoprotein; AGP, alpha-1 acid glycoprotein; ALT, alanine aminotransferase; AOL, Aspergillus oryzae *lectin; CHC, chronic hepatitis C; DSA,* Datura stramonium *lectin; GGT, gamma-glutamyltransferase; HA, hyaluronic acid; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; MAL,* Maackia amurensis *lectin; TIMP1, tissue inhibitors of metalloproteinases 1.*

From the [1]Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, Japan; [2]Research Center for Medical Glycoscience, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan; [3]Department of Virology & Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan; [4]Department of Internal Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [5]Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan; [6]Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan; [7]Pathology, School of Medicine, Keio University, Japan; [8]Department of Pathology, Kurume University School of Medicine, Japan.

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