一方，N市U地区コホートにおける検討では，HCV抗体陽性者の各HCV遺伝子配列はきわめて近いクラスターに分類されたことから，この地域のHCV感染がほぼ30年以上前に地域発生したことが明らかにされた[5]。

Ⅲ HCV持続感染者のALT値と肝組織像

N市U地区コホートでは，HCV RNA陽性者のALT値異常率と肝組織所見についての知見が得られた[6]。ALT値異常率は男性が72.3%(99人/137人)，女性が56.9%(112人/197人)であり，有意に女性においてALT値異常率が低かった。また，当時の犬山分類を用いた肝組織の検討では，ALT値正常43例の83.7%(36例)は慢性持続性肝炎(chronic persistent hepatitis；CPH)の所見であったが，ALT値が異常を示す105例ではその42.9%(45例)が慢性活動性肝炎(chronic active hepatitis；CAH)2Bあるいは肝硬変(liver cirrhosis；LC)であった(表2)。また50歳未満の男女を比較すると，男性のほうが肝組織像の進展が早いことが示された。

Ⅳ 10年間のHCV感染実態の変化

現在でもHCVには有効な感染予防手段がない。一方で，通常の日常生活ではC型肝炎の新規感染が起こりうるのかどうかについては不明であった。そこで，調査開始から10年経った2001年に同一地域の10年間に及ぶHCV感染実態の変遷を検討したところ，HCV抗体陽性率は1991年の33.1%から2001年には24.0%に低下していた[7]。とくにこの10年間で50歳代以下のHCV抗体陽性率の低下が顕著であったことから，この地域では新たな感染者は出現していないものと考えられた。

また，1991～2001年までの10年間にわたり161組の夫婦を追跡調査したところ，夫婦どちらかがHCV抗体陽性である27組の夫婦においては，10年間でその配偶者に新規HCV抗体陽性者は認められなかったことから，一般の日常生活においては，C型肝炎感染の危険性はき

表2 HCV RNAとALT値からみた肝組織所見

肝組織像	HCV RNA(−)(n=9)	HCV RNA(+)(n=148)	
		ALT値正常(n=43)	ALT値異常(n=105)
正常	8 (88.9%)	3 (7.0%)	0 (0.0%)
CPH	1 (11.1%)	36(83.7%)	15(14.3%)
CAH2A	0 (0.0%)	4 (9.3%)	45(42.9%)
CAH2B	0 (0.0%)	0 (0.0%)	30(28.6%)
LC	0 (0.0%)	0 (0.0%)	15(14.3%)

わめて低いことが明らかとなった。

V C型肝炎の経過と一塩基多型(SNPs)

われわれは，本コホート研究が当時の21世紀COE(center of excellence)プログラムに採択されたことを機に(拠点リーダー：河田純男先生)，C型肝炎の臨床経過に影響する遺伝子多型について探索することとした。現在行われているゲノムワイド関連解析(genome-wide association study：GWAS)ではなかったが，HCV感染後の宿主応答に関与する候補遺伝子の一塩基多型(single nucleotide polymorphisms；SNPs)を抽出したところ，HCV RNA持続陽性と関係する20 SNPs，およびHCVキャリアのALT値異常と関係する15 SNPsを2001年に見出し，HCV感染の経過にはさまざまな宿主因子が関与する可能性を報告した[8]。そのなかの1つに，形質転換増殖因子(transforming growth factor：TGF)-βのプロモーターSNP(-509T/C)があったが，このSNPは，TGF-β遺伝子多型で最もよく解析されているシグナルペプチド内のPro10Leu多型とほぼ完全に連鎖不均衡であり，-509Cアレルのホモはそれ以外のgenotypeに比べて自然緩解例が有意に多いことが判明した。Pro10/-509CはTGF-βの低発現のハプロタイプと考えられ，これらの結果からTGF-β低発現の宿主ではHCVの排除が起きやすいことが示唆された[9]。その後2009年に，C型慢性肝炎の抗ウイルス治療効果に関係すると報告された宿主因子インターロイキン(interleukin：IL)-28BのSNPが，本コホートにおいても自然治癒に強く関連していることがわかっている[10][11]。

VI ALT値正常HCVキャリアのALT値異常率

病院コホートを用いた研究では，HCV感染者ではたとえALT値が正常でも肝線維化が進んでいることは広く受け入れられているエビデンスであるが，われわれは2006年までの追跡調査をもとに，果たして肝機能正常(ALT値30 IU/L以下)のHCVキャリアではどの程度の頻度でALT値が持続的に正常であるかについて検討した。その結果，239人の肝機能正常例における肝機能異常(ALT値31 IU/L以上)出現率は42.3%に上ることが明らかとされ，とくに65歳未満および肥満者では，コホート追跡中に有意に肝機能異常率が高いことを報告した[12]。

地域コホートは病院コホートとは異なり肝機能検診受診者を対象としている性質上，採血間隔に限りはあるものの，2011年にはコホート開始から20年間のALT値の変動についてretrospectiveに解析した。1991～1995年までのスクリーニング期においてHCV RNA陽性であった600人のALT値をみてみると，20年間のうちにその53%がALT値31 IU/L以上の異常値となっていた[13]。このことから，HCVキャリアでは経時的な肝機能のフォローアップが

表3 自然陰性化に寄与する因子：多変量解析

因子	調整HR	95%CI	p値
AST 値正常(≦40 IU/L)	1.97	0.96～4.06	0.07
ZTT 値正常(≦11 Kunkel)	10.26	3.10～33.96	p<0.01
HBs 抗体陽性	2.22	1.19～4.13	0.01
HCV 抗体(C.O.I)	0.60	0.47～0.76	p<0.01

Cox 比例ハザードモデル
HR：ハザード比，95%CI：95%信頼区間，C.O.I：cut-off-index

必要であることが改めて示唆された。

VII ウイルス自然排除

これまで慢性持続感染が成立した後の HCV の生体からの自然排除の有無については不明であった。HCV 持続感染者のなかでウイルスの自然排除の有無とその頻度を解明することは感染自然史を理解するうえで重要である。そこで山形 HCV コホートの対象である 20 歳以上の 7,925 人中，HCV RNA 持続陽性であった 846 人において，HCV 感染の自然経過を探ることを目的に，コホート住民からのウイルス自然排除について解析した[14]。

逐年検診を 1 回以上受診し，抗ウイルス治療未治療例 475 人(平均 61.2±9.9 歳，平均観察期間 16.8±0.2 年)を対象に，経年的に TaqMan® HCV(ロシュ社)にて血中ウイルス量を測定したところ，Kaplan-Meier 法による血中 HCV RNA 累積自然陰性化率は 5 年で 4.3%，10 年で 5.1%，15 年で 7.1%であった。このデータをもとに人年法による解析を行ったところ，HCV キャリアはその自然経過の 0.6%/year person 程度の頻度で自然排除されている可能性があることが明らかとなった。さらに Cox 比例ハザードモデルを用いて HCV RNA 陰性化に関与する因子を分析したところ，ZTT が低値であることと HCV 抗体の cut-off-index が低力価であることが，HCV 自然排除と強く関係していた(表 3)。また松浦善治先生(大阪大学微生物病研究所)との共同研究にて，ウイルス自然排除例では HCV 中和結合(neutralization of binding；NOB)抗体が産生されていることを示した。これらの研究から，従来ウイルスが自然排除されることがほとんどないと考えられていた HCV 持続感染者においても，経過中に稀に自然排除される例があることが判明した。

VIII HCV 感染に伴う肝発癌・生命予後

HCV 感染者の予後の一端を明らかにする目的で，逐年検診受診者を対象に毎年腹部超音波検査を行っており，これまで 58 人の肝発癌が確認されている。内訳は genotype 1b が 69%，2b

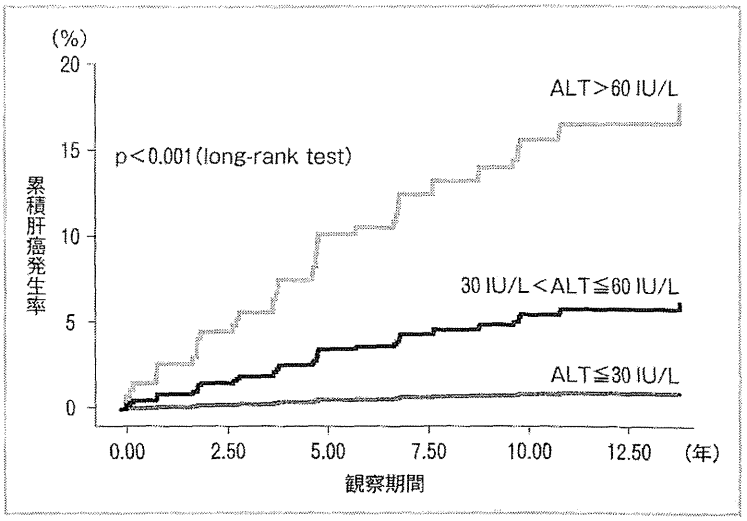

図2 初診時 ALT 値と肝発癌
Kaplan-Meier 法

表4 本コホートにおける肝発癌に寄与する因子

変量	HR(95%CI)*	p値*
男性	1.7(0.9~3.2)	0.077
ALT>60 IU/L	19.0(4.5~80.1)	<0.001
30 IU/L<ALT≦60 IU/L	6.3(1.4~28.2)	0.016
genotype 1b	2.4(1.2~4.8)	0.012

Cox 比例ハザードモデル
*：年齢，手術歴，輸血歴などで調整

(文献 15)より引用)

が 26%であり，2a からの発癌は認めなかった。発癌率を人年法でみてみると，1,000 人年あたり平均 5.1 人(男性 8.5 人，女性 3.5 人)と高い確率で発癌がみられることがわかった。また初診時 ALT 値で発癌率をみてみると，ALT 値 60 IU/L 以上および 30~60 IU/L の群で有意に肝発癌がみられ(図 2)，肝発癌に寄与する因子は男性，genotype 1b および ALT 値であった(表 4)[15]。

さらに，HCV 感染が最終転帰に及ぼす影響について，HCV 抗体陽性者 1,078 人全員を対象に，関連自治体の協力を得て，死亡診断書をもとにこの肝炎コホートにおける生命予後調査と死因調査を行った[13]。その結果，観察開始から平均 17.8 年の追跡では，HCV RNA 陽性者が陰性者に比べ肝疾患関連死の頻度が有意に高かった(13.4% vs. 4.5%；p<0.01)。

おわりに

これまで，住民検診をベースにした肝炎コホート設定とその追跡検診を行い，HCV 感染に

限らず，G型肝炎ウイルス[16)17)]やTTウイルス[18)]に関する分子疫学研究や臨床的意義について報告してきたが，そのいずれもがhospital-based studyでは得られないデータであった。20年にわたるわれわれのコホート研究からは，HCV感染の疫学，HCV感染経過と宿主因子の関係，キャリアにおけるウイルス自然排除と肝発癌など，わが国におけるHCV感染の未解決の問題点の一端が解明できたものと考える。同時にこれらの研究からは，病院コホート同様，慢性肝臓病予防のための感染住民への治療介入の必要性が改めて示唆された。わが国では欧米に比しHCV感染例の高齢化が指摘されており，その肝発癌率は高いものであることから，このような地域におけるHCVキャリアの病診連携の構築が一層望まれる。

一方では，ここ数年のGWASにより，信頼度の高いHCV感染関連遺伝子が複数みつかるようになった。これらの遺伝子多型は，報告されているような抗ウイルス治療効果予測因子であるばかりでなく，C型肝炎の病態に密接に関連している可能性がコホート研究からも明らかとなりつつある。近年のC型肝炎の病態解明や診断・治療法の進歩には目覚ましいものがあるが，これら地域コホート研究を通じて，HCV感染克服のための質の高いキーインフォメーションがさらに発信されることを期待したい。

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Hepatitis C Virus Genotype 2 May Not Be Detected by the Cobas AmpliPrep/Cobas TaqMan HCV Test, Version 1.0

Tsunamasa Watanabe,[a] Takako Inoue,[b] Yasushi Tanoue,[c] Hisato Maekawa,[c] Susumu Hamada-Tsutsumi,[a] Shinsho Yoshiba,[c] Yasuhito Tanaka[a,b]

Department of Virology and Liver Unit[a] and Department of Clinical Laboratory,[b] Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan; Department of Gastroenterology, Sempo Takanawa Hospital, Tokyo, Japan[c]

Accurate hepatitis C virus (HCV) RNA quantification is essential for the management and efficacy of treatment of chronic hepatitis C. The HCV RNA level is assessed using real-time PCR-based assays. Two highly sensitive commercial assays for HCV RNA quantification are available in many countries: the Roche Cobas AmpliPrep/Cobas TaqMan HCV assay (CAP/CTM HCV) (Roche Molecular Systems, Inc., Pleasanton, CA) and the Abbott RealTime HCV assay (ART HCV) (Abbott Molecular, Inc., Des Plaines, IL). Despite its good performance with most HCV strains, the CAP/CTM HCV test version 1.0 (v1.0) fails to detect genotype 4 strains with single nucleotide polymorphisms at positions 145 and 165 in the 5′ untranslated region (5′ UTR) (1). HCV genotype 4 is restricted to particular geographical areas, and many countries, including Japan, continue to use CAP/CTM HCV v1.0 to monitor HCV RNA quantification.

We report two Japanese patients with HCV genotype 2a in whom HCV RNA was undetectable by CAP/CTM HCV v1.0, although hepatitis C viremia was confirmed by the ART HCV test (4.0 and 5.0 $\log_{10}$ IU of HCV RNA/ml) and the Architect HCV core antigen assay (Abbott Diagnostics, Lake Forest, IL) (95 and 107 fmol/liter). This failure could be related to two or three substitutions in the putative binding site for the TaqMan probe (Fig. 1). The substitutions are at position 145, as described for HCV genotype 4 (1), and positions 158 and 169, which have not been reported previously.

Underestimation of HCV genotype 2 RNA by CAP/CTM HCV v1.0 has been reported previously (2), but failure to detect HCV genotype 2a RNA is critical as this genotype is the second most common HCV genotype. Recently, a second version of the assay, CAP/CTM HCV v2.0 (3), with redesigned primers and an addi-

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Address correspondence to Yasuhito Tanaka, ytanaka@med.nagoya-cu.ac.jp.
T.W. and T.I. contributed equally to this article.

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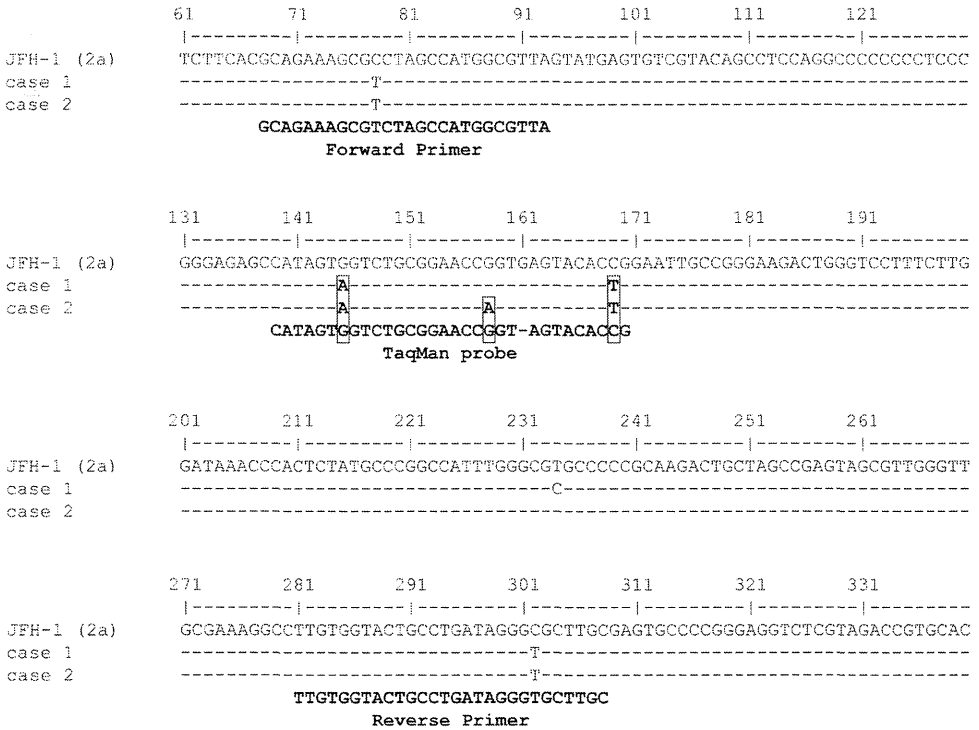

FIG 1 Alignment of HCV 5′-untranslated sequences for two patients with HCV genotype 2a, in whom HCV RNA was undetectable by the CAP/CTM HCV v1.0 assay, against the sequence of a reference HCV genotype 2a strain (JFH-1). Nucleotide substitutions were found in the patient's sequences compared to the reference sequence: G to A at position 145 and C to T at position 169 in both cases and G to A at position 158 in case 2, within the putative binding site of the TaqMan probe. Primer and probe sequences were obtained from the Japanese Patent Office (patent no. 4638388).

tional probe, has been released in Western Europe and the United States to resolve the problem of underestimation of HCV genotype 4 viral RNA (4). CAP/CTM HCV v2.0 detected and quantified the HCV genotype 2a RNA in the two specimens that were not detected by v1.0 at 4.17 $\log_{10}$ IU/ml and 5.05 $\log_{10}$ IU/ml. These values are comparable to that obtained by the Abbott RealTime HCV test. Clinicians need to be aware that the Roche Cobas TaqMan HCV test v1.0 may fail to provide a viral RNA result for genotype 2a, and if RNA results are discrepant with clinical findings, they need to confirm the HCV viral load using an alternative assay.

Nucleotide sequence accession numbers. The GenBank accession numbers for the nucleotide sequences are AB853937 for case 1 and AB853938 for case 2.

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Digestive Diseases

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Factors of Response to Pegylated Interferon/ Ribavirin Combination Therapy and Mechanism of Viral Clearance

Kayo Sugimoto[a] Soo Ryang Kim[b] Ahmed El-Shamy[c] Susumu Imoto[b] Kenji Ando[b] Ke Ih Kim[a] Yasuhito Tanaka[g] Yoshihiko Yano[d] Soo Ki Kim[h] Yutaka Hasegawa[e] Aya Fujinami[f] Mitsuhiro Ohta[f] Hatae Takashi[e] Hak Hotta[c] Yoshitake Hayashi[d] Masatoshi Kudo[i]

Departments of [a]Pharmacy and [b]Gastroenterology, Kobe Asahi Hospital, [c]Department of Microbiology and [d]Division for infectious Disease Pathology, Center for Infectious Diseases, Kobe University Graduate School of Medicine, and [e]Educational Center for Clinical Pharmacy and [f]Medical Biochemistry, Kobe Pharmaceutical University, Kobe, [g]Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, [h]Department of Gastroenterology, Kyoto University, Kyoto, and [i]Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osakasayama, Japan

Key Words
Viral clearance · Interferon λ1 · Pegylated interferon · Ribavirin · Interleukin 28B · Interferon and ribavirin resistance-determining region · Interferon sensitivity-determining region

Abstract
Objectives: This study explores viral factors of the interferon (IFN) and ribavirin (RBV) resistance-determining region (IRRDR), the IFN sensitivity-determining region (ISDR) and the core protein, and host factor interleukin 28B associated with response to pegylated IFN (PEG-IFN) and RBV combination therapy, and the correlation of viral and host factors with IFN-λ1. ***Methods:*** A total of 58 patients underwent PEG-IFN/RBV combination therapy for 48 weeks. The pretreatment factors associated with rapid virological response (RVR) and sustained virological response (SVR) were analyzed. Pretreatment IFN-λ1 serum levels were compared with the viral and host factors. ***Results:*** Univariate analysis showed that IRRDR ≥6 and ISDR ≥2 were significant pretreatment predictors of RVR, and multivariate analysis identified IRRDR ≥6 and hemoglobin as significant predictors of SVR. Pretreatment IFN-λ1 was significantly higher in the SVR group than in the non-SVR group and also in the IRRDR ≥6 group than in the IRRDR ≤5 group. ***Conclusions:*** IRRDR ≥6 was the only significant predictor of SVR and was correlated with IFN-λ1. High serum levels of IFN-λ1 may be conducive to effective PEG-IFN/RBV combination therapy because of the immunomodulatory system.

Introduction

Although the triple therapy of combined pegylated interferon (PEG-IFN), ribavirin (RBV) and protease inhibitors has already been initiated, PEG-IFN and RBV combination therapy for chronic hepatitis C virus (HCV) infection with a high viral load of genotype 1b, the standard

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Soo Ryang Kim, MD
Department of Gastroenterology
Kobe Asahi Hospital, 3-5-25 Bououji-cho, Nagata-ku
Kobe 653-0801 (Japan)
E-Mail asahi-hp@arion.ocn.ne.jp

treatment in Japan since 2004, provides sustained virological response (SVR) in only approximately 50% of such patients [1]. Single-nucleotide polymorphisms in proximity to the interleukin 28B (IL-28B) gene (rs8099917, rs12979860) on chromosome 19 is reported to be a host-related factor of virological response to PEG-IFN and RBV combination therapy [2–4]. In recent years, viral factors such as the core protein, nonstructural protein 5A (NS5A), the IFN sensitivity-determining region (ISDR) and the IFN/RBV resistance-determining region (IRRDR) [4–8] have been associated with virological response. Nonetheless, the mechanism of how these host and viral factors affect viral clearance has not been precisely elucidated to date.

IFN-λ1 is considered to be associated with the inhibition of the replication of HCV by an immunological mechanism [9, 10]. Few studies, however, have demonstrated the correlation among IFN-λ1 serum levels, the clinical outcome of PEG-IFN and RBV combination therapy, and viral and host factors. We investigated the viral and host factors associated with response to PEG-IFN and RBV combination therapy and the correlation of viral and host factors with IFN-λ1.

Table 1. Patient baseline characteristics

Age (years)	57.3±10.4
Sex (male/female)	34/24
BMI	22.6±3.8
HCV-RNA (log IU/ml)	6.0±0.6
ALT (U/l)	54.8±64.0
γ-GTP (U/l)	57.0±64.7
Hemoglobin (g/dl)	13.7±1.98
Platelets ($\times 10^4$/mm^3)	16.0±5.0
Total cholesterol (mg/dl)	174.0±34.0
IL-28B (major/minor)	43/15
IFN-λ1 (pg/ml)	31.0±24.2
ISDR (≥2/≤1)	14/44
IRRDR (≥6/≤5)	22/36
Core aa 70 (arginine/glutamine)	39/19
Core aa 91 (leucine/methionine)	39/19

Data are shown as number (n) or mean ± SD.

Patients and Methods

Patients

A total of 58 patients (32 men, 26 women; age 57.3 ± 10.4 years) seen at Kobe Asahi Hospital and diagnosed with chronic HCV and high viral loads of genotype 1b were enrolled in the study. Patients demonstrating hemoglobin levels ≥11 g/dl (women) or ≥12 g/dl (men), platelet count ≥9 × 10^4/mm^3, HCV RNA ≥5.0 log IU/ml, neutrophil count ≥1,500/mm^3 and thyroid-stimulating hormone levels within normal limits were included in the study; those demonstrating human immunodeficiency virus or hepatitis B coinfection, creatinine clearance <50 ml/min, liver disease other than chronic hepatitis C, evidence of advanced liver disease, preexisting psychiatric conditions, or a history of severe psychiatric disorder were excluded.

Treatment comprised PEG-IFN-α2b (1.5 μg per kilogram body weight, once a week) plus RBV (600–1,000 mg daily, based on body weight) for a total of 48 weeks, according to the standard treatment protocol for Japanese patients. Informed written consent was obtained from each patient and the study protocol conformed to the ethical guidelines approved by the Ethics Committee of Kobe Asahi Hospital.

Laboratory Tests

HCV RNA was extracted from 140 μl of serum with the use of a commercially available kit (QIAmp viral RNA kit; Qiagen, Tokyo, Japan). Amplification of full-length NS5A and the core regions of the HCV genome was carried out as described [5]. The sequences of the amplified fragments of NS5A and the core regions were determined by direct sequencing without subcloning. The amino acid (aa) sequences were deduced and aligned with the use of GENETYX Win software version 7.0 (GENETYX Corp., Tokyo, Japan). Genetic polymorphism rs8099917 around the IL-28B gene was determined by real-time PCR using the TaqMan assay. We defined the IL-28B major allele as homozygous (TT) for the major sequence and the minor allele as homozygous (GG) or heterozygous (TG) for the minor sequence. IFN-λ1 was assayed before initiation of therapy and at 4, 12 and 48 weeks after therapy by ELISA Ready-SET-Go (unit, pg/ml; NatuTec, Frankfurt, Germany).

Statistical Analysis

Rapid virological response (RVR) and SVR were defined as undetectable HCV RNA at weeks 4 and 24, respectively, after treatment. The potential pretreatment factors associated with virological response and comprising age, sex, BMI, HCV RNA load, alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), hemoglobin, platelets, IFN-λ1, single-nucleotide polymorphisms in the IL-28B gene region, mutations in NS5A – especially those in ISDR (ISDR ≥2 and ISDR ≤1) and IRRDR (IRRDR ≥6 and IRRDR ≤5) – and mutated core protein amino acid substitutions at aa 70 of arginine (Arg70), or glutamine (Gln70), and at aa 91 of leucine (Leu91), or methionine (Met91), were examined. Factors associated with virological response were assessed by univariate analysis using Student's t test, Fisher's exact test or χ^2 test, and by multivariate analysis using logistic regression analysis. The factors in multivariate logistic regression analysis were included in descending order according to correlativity. The most appropriate model was chosen by AIC (Akaike Information Criterion). We compared pretreatment IFN-λ1 in the IRRDR ≥6 and IRRDR ≤5 groups, in the ISDR ≥2 and ISDR ≤1 groups, in the IL-28B TT genotype for the major sequence and in the IL-28B GG genotype and TG genotype for the minor sequence, and in the core protein (aa 70 and aa 91) wild and mutant. Variables with a p value <0.05 were considered statistically significant. All statistical analyses were carried out with the use of Excel Statistics 2011 by SSRI.

Table 2. Correlation of baseline characteristics with clinical outcome of RVR and non-RVR

	RVR	Non-RVR	p value
Age (years)	48.6±6.2	58.2±10.7	0.054
Sex (male/female)	4/1	27/23	0.373
BMI	21.8±1.5	22.9±4.0	0.536
HCV-RNA (log IU/ml)	5.8±0.8	6.0±0.56	0.303
ALT (U/l)	141.4±178.9	47.2±36.2	0.304
γ-GTP (U/l)	99.6±67.9	49.7±61.0	0.090
Hemoglobin (g/dl)	14.0±1.7	13.7±1.8	0.707
Platelets ($\times 10^4$/mm^3)	16.0±5.8	16.0±4.8	0.982
Total cholesterol (mg/dl)	158±30.2	174.6±34.1	0.354
IL-28B (major/minor)	5/0	35/15	0.308
IFN-λ1 (pg/ml)	24.9±10.4	31.3±25.3	0.582
ISDR (≥2/≤1)	4/1	10/40	**0.012**
IRRDR (≥6/≤5)	5/0	15/35	**0.004**
Core aa 70 arginine/glutamine	3/2	32/16	1
Core aa 91 leucine/methionine	4/1	33/17	1

Data are shown as number (n) or mean ± SD. Bold p values are significant.

Table 3. Correlation of baseline characteristics with clinical outcome of SVR and non-SVR

	SVR	Non-SVR	p value
Age (years)	53.7±10.1	60.3±9.9	**0.015**
Sex (male/female)	20/6	14/18	**0.016**
BMI	23.0±3.7	22.3±3.9	0.484
HCV-RNA (log IU/ml)	5.9±0.7	6.1±0.5	0.300
ALT (U/l)	54.0±38.8	55.6±79.5	0.926
γ-GTP (U/l)	72.2±87.6	44.6±33.8	0.14
Hemoglobin (g/dl)	14.6±1.5	13.0±1.6	**<0.01**
Platelets ($\times 10^4$/mm^3)	17.1±4.8	15.2±5.0	0.135
Total cholesterol (mg/dl)	173.5±33.2	174.3±35.0	0.941
IL-28B (major/minor)	24/2	19/3	**0.006**
IFN-λ1 (pg/ml)	38.8±29.3	24.7±17.2	**0.037**
ISDR (≥2/≤1)	8/18	6/26	0.36
IRRDR (≥6/≤5)	18/8	4/28	**<0.01**
Core aa 70 arginine/glutamine	20/5	17/14	0.087
Core aa 91 leucine/methionine	19/7	20/12	0.412

Data are shown as number (n) or mean ± SD. Bold p values are significant.

Results

Patient baseline characteristics are listed in table 1. RVR was observed in 8.6% (5/58) and SVR in 44.8% (26/58) of the patients. ISDR ≥2 and IRRDR ≥6 were significantly associated with RVR as assessed by univariate analysis ($p = 0.012$, $p = 0.004$; table 2). IRRDR ≥6 was most significantly correlated with RVR, which was from biased data of distribution (table 2). As a result, we were not able to conduct multivariate analysis for RVR. By univariate analysis, the significant factors associated with SVR were age, sex, hemoglobin, IL-28B major, IRRDR ≥6 ($p = 0.015$, $p = 0.016$, $p < 0.001$, $p = 0.006$, $p < 0.001$, $p = 0.037$; table 3). The pretreatment IFN-λ1 serum level in SVR was significantly higher than in non-SVR (38.8 vs. 24.7 pg/ml, $p = 0.037$; table 3). By multivariate analysis, hemoglobin and IRRDR ≥6 were significantly associated with SVR ($p = 0.02$, $p = 0.005$; table 4). Pretreatment IFN-λ1 was significantly higher in the IRRDR ≥6 group than in the IRRDR ≤5 group (40.5 vs. 25.2 pg/ml, $p = 0.041$; fig. 1), but demonstrated no significant difference between the ISDR ≥2 group and the ISDR ≤1 group (37.2 vs. 29.1 pg/ml, $p = 0.45$; fig. 1), among the IL-28B TT genotype group, the TG genotype group and the GG genotype group (TT vs. TG, 33.4 vs. 24.6 pg/ml, $p = 0.26$; TT vs. GG, 33.4 vs. 20.8 pg/ml, $p = 0.48$; TG vs. GG, 24.6 vs. 20.8 pg/ml, $p = 0.82$; fig. 1), and between core protein wild and mutant of aa 70 and aa 91 (aa 70 wild vs. mutant, 30.8 vs. 30.0 pg/ml, $p = 0.91$; aa 91 wild vs. mutant, 34.6 vs. 23.7 pg/ml, $p = 0.05$; fig. 1).

Table 4. Factors associated with SVR by multivariate analysis

	Odds ratio	95% CI	p value
Age (years)	0.9247	0.85–1.01	0.0678
Sex	5.4742	0.44–68.64	0.1876
Hemoglobin	2.4704	1.12–5.43	**0.0244**
IL-28B (major/minor)	5.1960	0.60–45.10	0.1350
IFN-λ1 (pg/ml)	1.0230	0.98–1.07	0.2758
IRRDR (≥6/≤5)	16.9320	2.39–119.77	**0.0046**

Bold p values are significant.

Discussion

Pretreatment factors significantly and independently predictive of the outcome of treatment of patients infected with high viral loads of HCV-1b are IL-28B major genotype (TT) as a host factor [11], and substitutions of aa 70

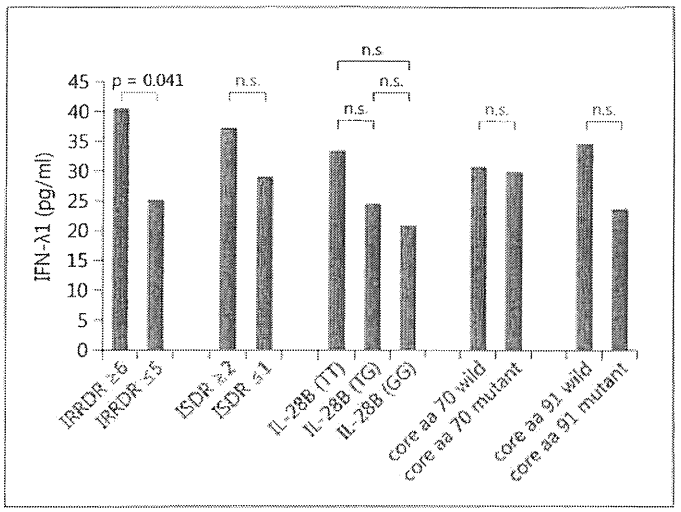

Fig. 1. Comparison of IFN-λ1 in the IRRDR ≥6 and IRRDR ≤5, ISDR ≥2 and ISDR ≤1, IL-28B, core aa 70 and core aa 91. Pretreatment IFN-λ1 was significantly higher in the IRRDR ≥6 group than in the IRRDR ≤5, but demonstrated no significant difference between the ISDR ≥2 group and the ISDR ≤1 group, among the IL-28B TT genotype group, the GG genotype group and the TG genotype group, between core protein wild and mutant of aa 70 and aa 91.

and aa 91 in the HCV core region, and high sequence variations in IRRDR (≥6) and in ISDR (≥2) as viral factors [4, 5, 7, 8, 11]. By univariate analysis, our study showed that ISDR and IRRDR were significant pretreatment predictors of RVR, and by multivariate analysis that IRRDR and hemoglobin were significant predictors of SVR. Because of the small number of RVR patients in our data, we were not able to carry out multivariate analysis for identifying RVR predictors. Our results support a previous study [12], and by univariate analysis we demonstrated a significant correlation between high pretreatment IFN-λ1 serum levels and SVR, but were unable to do so by multivariate analysis. On the other hand, although we were unable to demonstrate IL-28B as a predictor of SVR, some studies have demonstrated it as a positive predictive factor [2, 13, 14].

The level of IFN-λ1 has been reported to be significantly higher in carriers of the IL-28B major genotype than in those of the IL-28B homozygous minor sequence [9]. In the present study, the level of serum IFN-λ1 was higher in carriers of the IL-28B major genotype (TT) than in those of the IL-28B homozygous (GG) and the heterozygous (TG) minor sequence, but not significantly different ($p = 0.48$, $p = 0.26$). Because the number of carriers of the IL-28B homozygous allele (GG) was small (n = 2), we compared the level of serum IFN-λ1 in the IL-28B major homozygous allele (TT) and in the IL-28B minor homozygous (GG) as well as in the heterozygous (TG) allele. Nonetheless, for unclear reasons, no significant association was observed between a high level of serum IFN-λ1 and IL-28B major (major 33.4 pg/ml, minor 24.1 pg/ml; $p = 0.20$; data not shown). Further study is needed to clarify the relation between IL-28B and IFN-λ1.

It is well known that the antiviral mechanism of IFN comprises two phases [15, 16]. The first is direct inhibition of viral replication mediated by a number of proteins induced through the activation of the JAK-STAT pathway, including double-stranded RNA-activated protein kinase, myxovirus resistance gene A and 2′,5′-oligoadenylate synthetase, which block translation, block replication and degrade viral RNA, respectively [17–21]. The second is an indirect antiviral mechanism mediated by the stimulation of the host cell-mediated immune function including the cytotoxic T cell. The fact that IFN-λ1 significantly downregulates the secretion of IL-13 but elevates IFN-γ suggests that IFN-λ1 is related to an elevation of the Th1 response accompanied with a decrease of the Th2 response [10]. High levels of IFN-λ1 predispose to spontaneous resolution of HCV infection because of an elevation of the Th1 response [9]. Also, IFN-λ1 upregulates the chemokines MIG (Monokine induced by IFN-γ), IP-10 (IFN-γ-inducible protein 10) and I-TAC (IFN-inducible T cell α-chemoattractants), which are antimicrobial chemoattractants in peripheral blood mononuclear cells [22]. Taken together, these data suggest that IFN-λ1 stimulates the immunomodulatory effect [23].

The epitope located at position 2416, at a distance of 37 aa from IRRDR has been identified as an HLA-A26 CD8+ T cell epitope [24], which was targeted in all patients examined with acute resolving HCV infection. Therefore, IRRDR is regarded as the area (NS5A) related to immune function [5]. Our data demonstrated that IRRDR was significantly associated with IFN-λ1. From the above results, we infer that the achievement of SVR in patients with high IFN-λ1 levels is associated with the immunomodulatory system. Because of the small number of patients in our study, analysis in a large-scale multicenter study is needed.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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Prediction of Response to Pegylated Interferon/Ribavirin Combination Therapy for Chronic Hepatitis C Genotypes 2a and 2b and High Viral Load

Soo Ryang Kim[a] Ahmed El-Shamy[b, h] Susumu Imoto[a] Ke Ih Kim[a] Kayo Sugimoto[a] Soo Ki Kim[e] Yasuhito Tanaka[f] Takashi Hatae[c] Yutaka Hasegawa[c] Aya Fujinami[d] Mitsuhiro Ohta[d] Hak Hotta[b] Masatoshi Kudo[g]

[a]Department of Gastroenterology, Kobe Asahi Hospital, [b]Division of Microbiology, Center for Infectious Diseases, Kobe University Graduate School of Medicine, [c]Educational Center for Clinical Pharmacy and [d]Department of Medical Biochemistry, Kobe Pharmaceutical University, Kobe, [e]Department of Gastroenterology, Kyoto University, Kyoto, [f]Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, and [g]Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osakasayama, Japan; [h]Department of Virology, Faculty of Veterinary Medicine, Suez Canal University, Ismalia, Egypt

Key Words

Hepatitis C virus · Genotype 2a · Genotype 2b · IFN/RBV resistance-determining region · IL28B · Sustained virological response · Pegylated IFN/RBV

Abstract

Objective: We investigated the impact of host genetics represented by the single nucleotide polymorphism (SNP) of the IL28B gene and viral genetic variations within the nonstructural protein 5A (NS5A) [including the interferon (IFN)/ribavirin (RBV) resistance-determining region (IRRDR) and the IFN sensitivity-determining region (ISDR)] on the outcome of pegylated IFN and RBV (PEG-IFN/RBV) treatment. ***Methods:*** Sixty-six patients infected with hepatitis C virus (HCV)-2a or HCV-2b who received PEG-IFN/RBV for 24 weeks were examined. ***Results:*** In HCV-2a, the major genotype of IL28B SNP showed a tendency toward association with sustained virological response (SVR) and rapid virological response (RVR), and four or more mutations in IRRDR (IRRDR[2a] ≥4) and one or more mutations in ISDR plus its carboxyl-flanking region (ISDR/+C[2a] ≥1) were significantly associated with SVR and RVR. In HCV-2b, one or more mutations in the N-terminal part of IRRDR (IRRDR/N[2b] ≥1) were significantly associated with RVR. Multivariate analysis identified the major genotype of IL28B SNP and IRRDR[2a] ≥4 as independent predictive factors of SVR in HCV-2a, with IRRDR[2a] ≥4 being more powerful than the IL28B SNP. Also, IRRDR[2a] ≥4 in HCV-2a and IRRDR/N[2b] ≥1 in HCV-2b were significant determiners of RVR. ***Conclusion:*** The NS5A sequence heterogeneity and IL28B SNP are useful factors to predict the sensitivity to PEG-IFN/RBV therapy in HCV-2a and HCV-2b infections.

Introduction

In Japan, patients infected with hepatitis C virus (HCV) genotype 1b constitute about 70% of total HCV infection; the rest are infected with HCV-2a (25%) or HCV-2b (5%) [1]. The protease inhibitor, *telaprevir*, ap-

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Soo Ryang Kim, MD
Department of Gastroenterology, Kobe Asahi Hospital
3-5-25 Bouoji-cho, Nagata-ku
Kobe 653-0801 (Japan)
E-Mail asahi-hp@arion.ocn.ne.jp

proved in November 2011 by the Ministry of Health, Labor and Welfare, Japan, has shown sustained virological response (SVR) of more than 70% in HCV patients with high viral loads of genotype 1b [2].

Currently, combination therapy with pegylated interferon and ribavirin (PEG-IFN/RBV) is the standard treatment for chronic hepatitis C (CHC) patients infected with HCV-2a and HCV-2b. Patients infected with HCV genotypes 2 and 3 and treated with PEG-IFN/RBV show higher rates of SVR than those infected with HCV genotype 1 [3–5].

Sequence variations within a region in the nonstructural protein 5A (NS5A) of HCV-1b, defined as the IFN sensitivity-determining region (ISDR) [6] and the IFN/RBV resistance-determining region (IRRDR) [7], show correlation with IFN responsiveness.

In addition to the NS5A sequence variation, HCV core protein polymorphism has been proposed as a pretreatment predictor of poor virological response in HCV-1b-infected patients treated with PEG-IFN/RBV [8]. Host genetic factors associated with response to PEG-IFN/RBV therapy for HCV-1b and a high viral load are single nucleotide polymorphisms (SNPs) located in interleukin (IL)28B (rs8105790, rs11881222, rs8103142, rs28416813, rs4803219, rs8099917, rs7248668 and rs12979860) on chromosome 19 [9–12]. Moreover, on-treatment factors are mainly related to viral kinetics within the first few weeks of treatment [13].

At this stage, however, it is not clear whether NS5A sequence variation, including ISDR and IRRDR, core protein polymorphism, IL28B SNP and viral kinetics, are predictive of treatment outcome in HCV-2a and HCV-2b infections. In this context, we have recently reported that sequence heterogeneity within IRRDR of HCV-2a isolates (IRRDR[2a]) or within its N-terminus of HCV-2b isolates (IRRDR/N[2b]) is closely correlated with treatment responses, and that sequence heterogeneity within the ISDR plus its carboxyl-flanking region (ISDR/+C[2a]) is significantly associated with SVR in HCV-2a infection [14]. To further expand these observations in the present study, we investigated the effect of host genetics represented by IL28B SNP, the viral kinetics and the viral genetic heterogeneity in NS5A, and the core protein on the outcome of PEG-IFN/RBV treatment in HCV-2a and HCV-2b infections.

Materials and Methods

Patients

A total of 66 patients chronically infected with HCV-2a (n = 35) and HCV-2b (n = 31) seen at Kobe Asahi Hospital and Kobe University Hospital, Kobe, Japan, were enrolled in the study. The HCV subtype was determined according to the method of Okamoto et al. [15]. Inclusion and exclusion criteria were as follows, patients demonstrating hemoglobin levels ≥11 g/dl (women) or ≥12 g/dl (men), platelet counts $\geq 9 \times 10^4/mm^3$, HCV RNA ≥5.0 log IU/ml, neutrophil count $\geq 1{,}500/mm^3$ and thyroid-stimulating hormone levels within normal limits were included in the study; those demonstrating human immunodeficiency virus or hepatitis B virus coinfection, creatinine clearance <50 ml/min, cause of liver disease other than CHC, evidence of advanced liver disease, pre-existing psychiatric conditions or a history of severe psychiatric disorder were excluded. All of the patients were treated with PEG-IFN α-2b (Pegintron®; Schering-Plough, Kenilworth, N.J., USA; 1.5 mg per kg body weight, once a week, subcutaneously) and RBV (Rebetol®; Schering-Plough; 600–800 mg daily, per os), for 24 weeks according to the standard treatment protocol for Japanese patients established by the hepatitis study group of the Ministry of Health, Labour and Welfare, Japan. The patients received >80% of the scheduled dosage of PEG-IFN and RBV. Serum samples were collected at intervals of 4 weeks before, during and after the treatment and tested for HCV RNA and core antigen titers as described [16].

Table 1. Proportion of various virological responses of HCV-2a- and HCV-2b-infected patients to PEG-IFN/RBV treatment

Response	Proportion		
	All (n = 66)	HCV-2a (n = 35)	HCV-2b (n = 31)
RVR	44 (67)	23 (66)	21 (68)
Non-RVR	22 (33)	12 (34)	10 (32)
EVR	62 (94)	34 (97)	28 (90)
ETR	63 (95)	34 (97)	29 (94)
SVR	48 (73)	28 (80)	20 (65)
Non-SVR	18 (27)	7 (20)	11 (35)

Figures in parentheses are percentages.

Genetic Variation near the IL28B Gene

Genetic polymorphism rs8099917 around the IL28B gene was determined by real-time polymerase chain reaction (PCR) with the TaqMan assay (Roche Diagnostics, Tokyo, Japan) [9]. We defined the IL28B major allele as homozygous for the major sequence (TT) and the IL28B minor allele as homozygous (GG) or heterozygous (TG) for the minor sequence.

Viral Kinetics

The amount of HCV RNA was examined by the COBAS TaqMan HCV test. The patients who cleared HCV viremia (less than 17 IU/ml) by week 4 were defined as achieving rapid virological response (RVR). The amount of HCV core antigen was assessed by the IRM assay (Ortho Clinical Diagnostics, Tokyo, Japan) that provides a good correlation between the amount of HCV core antigen and the amount of HCV RNA, as described [17]. The HCV core antigen was measured on days 0 and 7 (week 1) according to the detection limit of 20 fmol/l established by the manufacturer. Early viral drop was defined as an HCV core antigen level of less than 20 fmol/l.

Table 2. Demographic characteristics of HCV-2a- and HCV-2b-infected patients with SVR and non-SVR

Factor	All	HCV-2a			HCV-2b		
		SVR	non-SVR	p value (SVR vs. non-SVR)	SVR	non-SVR	p value (SVR vs. non-SVR)
n	66	28	7	–	20	11	–
Age, years	52.0±13.4	50.6±14.6	57.3±10.1	0.2006	50.6±13.6	54.6±10.2	0.5910
Sex (male/female)	39/27	15/13	4/3	1.0000	11/9	9/2	0.2409
Body weight, kg	60.4±8.9	58.6±8.7	60.0±10.4	0.7100	59.6±9.2	66.9±2.5	**0.0056**
Platelets, $\times 10^4/mm^3$	18.0±5.9	19.0±5.3	15.2±5.6	0.1170	18.1±5.9	17.1±7.2	0.7412
Hemoglobin, g/dl	13.9±1.8	13.7±1.8	14.5±1.8	0.3641	13.8±1.7	14.4±1.8	0.5351
γ-GTP, U/l	47.4±47.6	35.0±29.9	45.3±29.5	0.2829	34.2±29.4	104.7±74.5	**0.0004**
ALT, U/l	56.8±64.0	47.6±38.3	55.4±41.3	0.5223	43.8±37.4	105.0±124.7	**0.0369**
HCV RNA, log IU/ml	6.1±0.8	5.9±0.6	5.7±1.0	0.8147	6.3±0.8	6.5±0.4	0.8042
HCV core antigen, fmol/l	7,659.0±6,852.9	6,638.8±6,489.3	5,901.5±5,963.0	0.8469	7,751.2±6,394.2	10,175.5±8,464.5	0.4776
Response (RVR/non-RVR)	44/22	21/7	2/5	**0.0331**	16/4	5/6	0.1055
IL28B genotype (major/minor)	60/6	26/2	5/2	0.1710	19/1	10/1	1.0000

Bold p values are significant.

Sequence Analysis of the NS5A and the Core Regions

Sequence analysis of the NS5A and the core regions of HCV was carried out as described [14, 16, 18]. In brief, RNA extracted from serum was reverse transcribed and amplified for NS5A and the core regions of the HCV genome; the resultant RT-PCR products were then subjected to a second round of PCR. The sequences of the amplified fragments were determined by direct sequencing without subcloning. The amino acid (aa) sequences were deduced and aligned. The aa residues of HCV-2a and HCV-2b isolates were numbered according to the polyprotein of HCV-J6 [19] and HCV-J8 [20], respectively.

Statistical Analysis

Statistical differences in treatment response according to patient baseline parameters of age, body weight, platelets, hemoglobin, γ-glutamyl transpeptidase (γ-GTP), alanine aminotransferase (ALT), HCV RNA load and HCV core antigen were determined by the Mann-Whitney U test for numerical variables and Fisher's exact probability test for categorical variables. Similarly, statistical differences in treatment response according to NS5A and genetic variation near the IL28B gene (genotype TT) were determined by Fisher's exact probability test. Multicolinearity was tested using Spearman's correlation. Spearman's rank correlation analysis was used to test for multicolinearity among candidate variables in the multivariable analysis. When correlation was >0.5, only one of the correlated variables was used in the logistic regression model.

Results

Patient Response to PEG-IFN/RBV Combination Therapy for HCV-2a and HCV-2b Infections

Among the 35 patients infected with HCV-2a, RVR at week 4 was achieved by 66% (23/35), early virological response (EVR) at week 12 by 97% (34/35) and end-of-treatment response (ETR) by 97% (34/35). Similarly, among the 31 infected with HCV-2b, RVR was achieved by 68% (21/31), EVR by 90% (28/31) and ETR by 94% (29/31). SVR was achieved by 28 (80%) HCV-2a patients and by 20 (65%) HCV-2b patients. Only 7 (20%) HCV-2a and 11 (35%) HCV-2b patients were non-SVR. No null-response (continuous viremia throughout the treatment and follow-up periods) was observed since all the non-SVR patients achieved HCV-RNA negativity at a certain point in time followed by a rebound in viremia either before or after the treatment course (relapse; table 1).

Patient baseline demographic characteristics and clinical and treatment response are shown in table 2. Among HCV-2a patients, no significant difference was observed between SVR and non-SVR. Among HCV-2b patients, on the other hand, lighter body weight, and lower γ-GTP and ALT levels showed a significant difference between SVR and non-SVR patients.

Correlation between IL28B and SVR or RVR

The frequency of allele rs8099917 among HCV-2a patients was 89% for the IL28B major genotype (TT; 31/35) and 11% for the minor genotype (non-TT; 4/35); among HCV-2b patients it was 94% (29/31) for TT and 6% (2/31) for non-TT. Among HCV-2a patients with the IL28B major genotype, SVR was achieved by 84% (26/31; p = 0.1710) and RVR by 71% (22/31; p = 0.1061; table 3). Among HCV-2b patients, on the other hand, SVR was achieved by 66% (19/29; p = 1.0000) and RVR by 69% (20/29; p = 1.0000; table 3). Thus, there was a tendency toward SVR and RVR in HCV-2a patients with the IL28B

Table 3. Correlation between IL28B genotype and SVR or RVR in HCV-2a and HCV-2b infections

		SVR	Non-SVR	p value	RVR	Non-RVR	p value
HCV-2a	IL28B (major; n = 31)	26 (84)	5 (16)	0.1710	22 (71)	9 (29)	0.1061
	IL28B (nonmajor; n = 4)	2 (50)	2 (50)		1 (25)	3 (75)	
HCV-2b	IL28B (major; n = 29)	19 (66)	10 (34)	1.0000	20 (69)	9 (31)	1.0000
	IL28B (nonmajor; n = 2)	1 (50)	1 (50)		1 (50)	1 (50)	

Figures in parentheses are percentages.

Table 4. Correlation between IL28B genotype and SVR or RVR according to IRRDR[2a] ≥4 or IRRDR[2a] ≤3 in HCV-2a infection

		SVR	Non-SVR	p value	RVR	Non-RVR	p value
IRRDR ≥4	IL28B (major; n = 21)	20 (95)	1 (5)	0.1700	19 (90)	2 (9)	0.2490
	IL28B (nonmajor; n = 2)	1 (50)	1 (50)		1 (50)	1 (50)	
IRRDR ≤3	IL28B (major; n = 10)	6 (60)	4 (40)	1.0000	3 (30)	7 (70)	1.0000
	IL28B (nonmajor; n = 2)	1 (50)	1 (50)		0	2 (100)	

Figures in parentheses are percentages.

major genotype, although the difference was not statistically significant, probably due to the small number of the patients examined. Moreover, among HCV-2a patients with the IL28B major genotype, SVR was achieved by 95% (20/21; p = 0.1700) and RVR by 90% (19/21; p = 0.2490) when involving IRRDR with 4 or more mutations (IRRDR[2a] ≥4) while SVR was achieved by 60.0% (6/10; p = 1.0000) and RVR by 30.0% (3/10; p = 1.0000) when involving of IRRDR with 3 or less mutations (IRRDR[2a] ≤3; table 4). Thus, there was a tendency toward SVR and RVR in HCV-2a patients with the IL28B major genotype when involving of IRRDR[2a] ≥4, but not IRRDR[2a] ≤3.

Correlation between Viral Kinetics Including Early Viral Drop and SVR

SVR was achieved by 91% (21/23) of HCV-2a-infected patients who achieved RVR, but by only 9% (2/23) of those who did not achieve RVR (table 5). Thus, RVR was significantly associated with SVR in HCV-2a infection (p = 0.0331). On the other hand, RVR was not significantly associated with SVR in HCV-2b infection (p = 0.1055; table 5).

HCV core antigen titers were measured one week after the initiation of treatment in 91% (32/35) and 97% (30/31) of patients infected with HCV-2a and HCV-2b, respectively. Patients with the HCV core antigen titer of <20 fmol/l after 1 week were considered as achieving early viral drop.

Table 5. Correlation between RVR and SVR in HCV-2a and HCV-2b infections

		SVR	Non-SVR	p value
HCV-2a	RVR (n = 23)	21 (91)	2 (9)	**0.0331**
	Non-RVR (n = 12)	7 (58)	5 (42)	
HCV-2b	RVR (n = 21)	16 (76)	5 (24)	0.1055
	Non-RVR (n = 10)	4 (40)	6 (60)	

Figures in parentheses are percentages.
Bold p values are significant.

As shown in table 6, RVR was achieved by 93% (13/14) of HCV-2a-infected patients who achieved early viral drop, and by only 44% (8/18) of those who did not. Thus, the early viral drop was significantly associated with RVR (p = 0.0075) but not with SVR (p = 0.1959) in HCV-2a infection. On the other hand, early viral drop was not associated with either RVR (p = 0.1405) or SVR (p = 0.6328) in HCV-2b infection.

Sequence Analysis of NS5A of HCV-2a and HCV-2b

Alignment of all the NS5A sequences of the HCV-2a genome obtained from pretreatment sera against the consensus sequences previously reported [14] revealed that

Table 6. Correlation between early viral drop and SVR or RVR in HCV-2a and HCV-2b infections

		SVR	Non-SVR	p value	RVR	Non-RVR	p value
HCV-2a	HCV core antigen <20 fmol/l after 1 week (n = 14)	13 (93)	1 (7)	0.1959	13 (93)	1 (7)	**0.0075**
	HCV core antigen ≥20 fmol/l after 1 week (n = 18)	13 (72)	5 (28)		8 (44)	10 (56)	
HCV-2b	HCV core antigen <20 fmol/l after 1 week (n = 6)	5 (83)	1 (17)	0.6328	6 (100)	0	0.1405
	HCV core antigen ≥20 fmol/l after 1 week (n = 24)	15 (63)	9 (37)		15 (63)	9 (37)	

Figures in parentheses are percentages. Bold p values are significant.

Table 7. Average number of aa mutations within IRRDR[2a], ISDR/+C[2a] and IRRDR/N[2b] of HCV NS5A obtained from pretreatment sera of HCV-2a- and HCV-2b-infected patients with SVR, non-SVR, RVR and non-RVR

NS5A region	Mutations, n					
	SVR	non-SVR	p value	RVR	non-RVR	p value
IRRDR[2a] (aa 2332–2387)	6.0±3.9	3.0±1.2	**0.0361**	6.9±3.7	2.7±1.3	**0.0003**
ISDR/+C[2a] (aa 2232–2262)	1.8±2.4	0±0	**0.0015**	2.0±2.5	0.4±0.6	**0.0107**
IRRDR/N[2b] (aa 2332–2357)	2.2±1.6	1.4±1.5	0.1578	2.6±1.4	0.4±0.5	**0.0002**

Bold p values are significant.

Table 8. Correlation between NS5A sequence heterogeneity and SVR or RVR in HCV-2a and HCV-2b infections

Factor	SVR	Non-SVR	p value	RVR	Non-RVR	p value
IRRDR[2a] ≥4 (n = 23)	21 (91)	2 (9)	**0.0331**	20 (87)	3 (13)	**0.0005**
IRRDR[2a] ≤3 (n = 12)	7 (58)	5 (42)		3 (25)	9 (75)	
ISDR/+C[2a] ≥1 (n = 21)	21 (100)	0	**0.0005**	17 (81)	4 (19)	**0.0313**
ISDR/+C[2a] = 0 (n = 14)	7 (50)	7(50)		6 (43)	8 (57)	
IRRDR/N[2b] ≥1 (n = 23)	17 (74)	6 (26)	0.0947	19 (83)	4 (17)	**0.0059**
IRRDR/N[2b] = 0 (n = 8)	3 (37)	5 (63)		2 (25)	6 (75)	

Figures in parentheses are percentages. Bold p values are significant.

the average number of aa mutations in IRRDR[2a] (RVR = 6.9 ± 3.7 vs. non-RVR = 2.7 ± 1.3; p = 0.0003) and ISDR/+C[2a] (2.0 ± 2.5 vs. 0.4 ± 0.6; p = 0.0107) was significantly larger in the isolates from RVR than in those from non-RVR patients. More importantly, the average number of aa mutations in IRRDR[2a] (SVR = 6.0 ± 3.9 vs. non-SVR = 3.0 ± 1.2; p = 0.0361) and ISDR/+C[2a] (1.8 ± 2.4 vs. 0 ± 0; p = 0.0015) was significantly larger in SVR than in non-SVR.

Similarly, alignment of all the NS5A sequences of the HCV-2b genome against the consensus sequences [14] revealed that the average number of aa mutations in IRRDR/N[2b] was significantly larger in RVR than in non-RVR (2.6 ± 1.4 vs. 0.4 ± 0.5; p = 0.0002); however, no significant difference was observed between SVR and non-SVR (table 7).

Correlation between NS5A Sequence Heterogeneity and SVR or RVR in HCV-2a and HCV-2b Infections

Ninety-one percent (21/23) of HCV-2a-infected patients with IRRDR[2a] ≥4 achieved SVR compared to only 58% (7/12) of those with IRRDR[2a] ≤3 (table 8). This result suggests that IRRDR[2a] ≥4 was significantly associated with SVR (p = 0.0331). Similarly, 87% (20/23) of IRRDR[2a] ≥4, but only 25% (3/12) of IRRDR[2a] ≤3, achieved RVR, with the result suggest-

ing that IRRDR[2a] ≥4 was also significantly associated with RVR (p = 0.0005; table 8).

Likewise, all of the 21 patients infected with HCV-2a having one or more mutations in ISDR/+C (ISDR/+C[2a] ≥1), but only 50% (7/14) of those with ISDR/+C[2a] = 0, achieved SVR, with the result suggesting that ISDR/+C[2a] ≥1 was significantly associated with SVR (p = 0.0005). The ISDR/+C[2a] heterogeneity was also significantly associated with RVR (p = 0.0313).

In HCV-2b infection, 83% (19/23) of patients with IRRDR/N[2b] ≥1 achieved RVR whereas 25% (2/8) of those with IRRDR/N[2b] = 0 achieved RVR (p = 0.0059). Thus, IRRDR/N[2b] ≥1 was significantly associated with RVR, but not with SVR (table 8).

Correlation between HCV Core Protein Sequence Heterogeneity and RVR or SVR

A close correlation between HCV core protein sequence patterns and treatment outcome has been proposed in HCV-1b infection [17, 21]; however, this information is still unclear in HCV-2a and HCV-2b infections. Therefore, in the current study, core regions were obtained from the pretreated sera and the aa sequences deduced and aligned with prototype sequences (HCV-J6 [19] and HCV-J8 [20]). Contrary to reports on HCV-1b infection [8], the residues at positions 70 and 91 are well conserved among HCV-2a and HCV-2b isolates, suggesting no correlation between treatment outcome and these observed residues [14].

Identification of Independent Factors Predictive of SVR in HCV-2a and HCV-2b Infections

To identify significant independent predictors of SVR in HCV-2a and HCV-2b infections, univariate and multivariate logistic regression analyses were carried out using all available data on patient baseline parameters and viral genetic polymorphic factors. In HCV-2a infection, univariate analysis identified two factors, the heterogeneity of IRRDR[2a] (≥4 vs. ≤3) and RVR, that were significantly associated with SVR. Subsequently, multivariate regression analysis including these factors revealed that IRRDR[2a] heterogeneity (p = 0.0264) platelets (p = 0.0369) and IL28B major genotype (p = 0.0424) were independent factors predictive of SVR (table 9).

In HCV-2b infection, univariate analysis identified two factors, body weight and γ-GTP levels, that were significantly associated with SVR. In the subsequent multivariate analysis, only the γ-GTP level (p = 0.0287) was identified as an independent factor predictive of SVR in

Table 9. Univariate and multivariate analyses of factors associated with SVR

	HCV-2a				HCV-2b			
	univariate		multivariate		univariate		multivariate	
	odds ratio (95% CI)	p value	odds ratio (95% CI)	p value	odds ratio (95% CI)	p value	odds ratio (95% CI)	p value
Age (years)	0.9643 (0.9017–1.0293)	0.2692			0.9722 (0.9127–1.0355)	0.3807		
Sex (male/female)	0.8654 (0.1627–4.6019)	0.8653			0.2716 (0.0464–1.5904)	0.1483		
Body weight (kg)	0.9830 (0.8960–1.0783)	0.7161			0.8610 (0.7492–0.9896)	**0.0350**		
Platelets ($\times 10^4$/mm^3)	1.1375 (0.9692–1.3350)	0.1147	1.3364 (1.0178–1.7548)	**0.0369**	1.0281 (0.9112–1.1599)	0.6531		
Hemoglobin (g/dl)	0.7761 (0.4921–1.2240)	0.2755			0.7964 (0.5051–1.2557)	0.3271		
γ-GTP (U/l)	0.9900 (0.9658–1.0148)	0.4264			0.9643 (0.9353–0.9942)	**0.0198**	0.8962 (0.8124–0.9887)	**0.0287**
ALT (U/l)	0.9953 (0.9759–1.0150)	0.6361			0.9836 (0.9645–1.0031)	0.0985		
HCV RNA (log IU/ml)	1.4501 (0.4960–4.2393)	0.4972			0.6931 (0.2145–2.2393)	0.5401		
HCV core antigen (fmol/l)	1.0000 (0.9999–1.0002)	0.7996			1.0000 (0.9998–1.0001)	0.3710		
IL28B major genotype	5.2000 (0.5871–46.0546)	0.1385	31.2194 (1.1256–865.8969)	**0.0424**	1.900 (0.1071–33.6995)	0.6618		
IRRDR[2a] mutations ≥4	7.5000 (1.1798–47.6758)	**0.0327**	15.3487 (1.3773–171.0478)	**0.0264**	–	–		
IRRDR/N[2b] mutations ≥1	–	–			4.7222 (0.8564–26.0395)	0.0748	36.2264 (0.9676–1,356.2272)	0.0521
Response (RVR/non-RVR)	7.5000 (1.1798–47.6758)	**0.0327**			4.8000 (0.9544–24.1396)	0.0570		

Bold p values are significant.

HCV-2b infection. The heterogeneity of IRRDR/N[2b], a viral factor, was identified as significantly associated with RVR (p = 0.0064; data not shown) but not with SVR (p = 0.0521; table 9).

Discussion

Host factors (such as age, sex, ethnicity, platelets, liver fibrosis and obesity) have been associated with the outcome of PEG-IFN/RBV therapy [22] for HCV genotype 1. Also, the clinical outcome of this therapy for HCV infection is influenced by a number of host factors such as IL28B [9–12] and viral factors including ISDR [17, 23] and core mutations at position 70 in genotype 1 [8].

We have previously compared the impact of IRRDR, ISDR and core mutations as viral genetic polymorphisms, and the IL28B genotype as a host genetic factor, in the clinical outcome of PEG-IFN/RBV therapy – SVR, relapse and nonvirological response (NVR) – for HCV-1b with a high viral load in Japanese patients. IRRDR ≥6 was identified as a viral genetic polymorphism that independently predicts SVR to PEG-IFN/RBV treatment [7, 16, 17, 24]. The IL28B minor genotype was identified as a host genetic factor that most effectively predicts NVR [24]. On the other hand, ISDR was identified as a factor showing significant correlation with RVR in our previous study, although it has been considered a viral determiner of SVR [22, 23].

To date, except for ours [24], few factors, including host and viral factors, and viral kinetics, have been shown to predict the outcome of PEG-IFN RBV therapy for HCV-2a and HCV-2b [25]. In the present study, multivariate analysis identified platelets (OR 1.3364; p = 0.0369), IL28B major genotype (OR 31.2194; p = 0.0424) and IRRDR[2a] ≥4 (OR 15.3487; p = 0.0264), on the one hand, and γ-GTP (OR 0.8962, p = 0.0287) on the other, as factors predictive of SVR in patients infected with HCV-2a and HCV-2b, respectively. Similarly, IRRDR[2a] ≥4 (OR 23.8493; p = 0.0014) and IRRDR/N[2b] ≥1 (OR 44.7766; p = 0.064) were identified as predictive of RVR in patients infected with HCV-2a and HCV-2b, respectively. Moreover, univariate analysis showed that ISDR/+C[2a] ≥1 was significantly associated with RVR.

Consistent with previous observations of HCV-1b, we have here demonstrated that in Japanese patients sequence heterogeneity within IRRDR is closely correlated with the treatment response of HCV-2a and HCV-2b infections, and that the IL28B major genotype is significantly associated with SVR in HCV-2a infection. Nonetheless, the effect of IL28B SNP appeared to be weaker in HCV-2a and HCV-2b infections than that seen in HCV-1b infections. In HCV-2a, ISDR/+C[2a] was considered a factor related to at least early viral dynamics as shown in HCV-1b. A mutation at position 70 of the core protein of HCV-1b has been correlated with PEG-IFN/RBV treatment outcome [8]. In the present study, however, we found no significant correlation between core protein polymorphism and treatment outcome in HCV-2a or HCV-2b infection. The sequence conservation observed at position 70 might be the reason for the lack of significant correlation between core protein polymorphism and treatment outcome in HCV-2a and HCV-2b infections.

For the correlation between viral kinetics and treatment outcome, we demonstrated that RVR was related to SVR (p = 0.0327) in HCV-2a infection; however, we were not able to relate RVR to SVR by multivariate analysis. Furthermore, early viral drop was related to only RVR (p = 0.0075) in HCV-2a infection.

In conclusion, the present results suggest the clinical usefulness of the sequence heterogeneity of NS5A in HCV-2a (IRRDR[2a] ≥4) and IL28B SNP for determining viral sensitivity to PEG-IFN/RBV therapy given to HCV-2a patients. A large-scale multicenter study is needed to clarify our conclusions.

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Disclosure Statement

The authors have no disclosures to make.

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