●まとめ

脂肪肝は症状がなく，健診においても経過観察されていることが多い．しかし，①一部には進行性で肝硬変や肝癌が合併する症例があること，②肝臓での脂肪蓄積は，インスリン抵抗性を惹起し，メタボリックシンドロームにおいては上流に位置すること，を念頭に，肝臓のみならず，糖尿病・脂質異常症・高血圧・虚血性心疾患など全身の疾患との関連でとらえていく必要がある．飽食の時代となり肥満人口の増加を背景に，脂肪肝の管理の強化が生活習慣病予防に貢献するものと考える．

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（清家正隆）

E CKD

1●糖尿病腎症とCKD

わが国の慢性透析療法の現況をみると，透析人口は増加の一途をたどり290,675人となっている(2009年12月31日現在)．なかでも，糖尿病腎症からの新規導入症例が44.5%と，1998年から第1位を占めている[1]．つまり，CKD(chronic kidney disease，慢性腎臓病)の主たる疾患が糖尿病腎症である．したがって，糖尿病腎症にCKDの概念を含んだ診断基準(私案)と，糖尿病腎症の早期診断と適切な治療によって，糖尿病腎症の病期寛解が得られ，かつ心血管疾患の発症を抑制できることの理解が重要である．

2●糖尿病腎症とCKDの病期分類の違い

糖尿病腎症の病期分類に関しては，日本糖尿病学会および日本腎臓学会の糖尿病腎症に関する合同委員会において，臨床的特徴である尿アルブミン量および尿タンパク量を主として作成された(表Ⅰ-9，図Ⅰ-20)．一方，CKD病期は主に推算糸球体濾過値(estimated glomerular filtration rate : eGFR)を基に分類されている(表Ⅰ-10)．

糖尿病腎症の病期分類では，腎機能に関して顕性腎症前期(第3期-A)，後期(第3期-B)の特徴として，GFR(Ccr)がそれぞれ「ほぼ正常」「低下」と記載されている．しかし，日常診療においては，正常アルブミン尿あるいは微量アルブミン尿期の糖尿病患者においても，GFR(Ccr)が低下している

14 病態別治療⑤ [脂肪肝] について

GL 62頁

Guideline/Guidance　ガイドラインの考え方

(1) ポイント

①近年，生活習慣の欧米化に伴い脂肪肝は増加しています

②脂肪肝の成因は多彩ですが，多くは肥満に伴う過栄養性です。単純性脂肪肝に対して脂肪性肝炎という病態が存在します。インスリン抵抗性と酸化ストレスにより進行性で，肝硬変症や肝癌に至る病態です

③減量が重要です

④基本的には2～4週間に1kgのペースで現体重の5％減を目標にします

(2) 疫　学

▶検診受診者の20～30％は脂肪肝を伴っており，年々増加しています。

▶脂肪肝は男性に多く，30～50歳までの約20％以上に合併しています。

▶女性は閉経後に増加してきます。

▶脂肪肝では肥満を約70％以上伴います。脂肪肝では脂質代謝異常，高血圧症，高血糖をそれぞれ約50％，約30％，約30％合併し，メタボリックシンドロームの合併は約30％とされています。

▶生活習慣の欧米化に伴い，ますます増加が予想されます。

Case/Variation　臨床でしばしば遭遇する状況

▶なぜ治療が必要か？――脂肪肝の場合，症状がなく検診で指摘されることが多いので治療の動機づけが難しいという現状があります。しかし，以下の点で治療が必要であることを認識する必要があります。

①脂肪肝の一部に脂肪性肝炎(non-alcoholic steatohepatitis：NASH)という進行性の病態があり，放置すると肝硬変症や肝癌を発症することがあります。最近，NASHが原因の肝癌が徐々に増加しています

1 脂肪性肝炎の病態

②脂肪肝はメタボリックシンドロームの肝臓での表現型で，高血圧や脂質異常症，糖尿病などの発症の上流に位置します。脂肪肝が改善することにより，インスリン抵抗性が改善し，心血管

2 脂肪肝はメタボリックドミノの上流

3 脂肪肝の診断

イベントの減少に寄与します

▶脂肪肝の評価はどうするか？ 腹部超音波検査などの画像診断で行う？ それとも実際に肝生検による病理組織検査で行うか？——脂肪肝は増悪を繰り返す疾患で長期の管理が必要です。

▶ウイルス性肝炎をはじめとした従来の肝疾患に比し肝癌発生率も低いし，心血管系のイベントが多いと言っても，遠い先のことだし…こうした点から，患者や医療者側の切実感が乏しいことがあります。ではどうすればよいでしょう？

4 脂肪肝と脂肪性肝炎（NASH）の鑑別

5 脂肪肝の治療

6 治療目標：ALT値が正常の脂肪肝に対して治療が必要か

▶Method／Approach 解法・診療の進め方

1 脂肪性肝炎の病態

▶最近注目されている脂肪性肝炎（non-alcoholic steatohepatitis：NASH）はインスリン抵抗性に加え，酸化ストレスが加わることにより発症すると考えられています[1]。この経過は，いわゆるtwo hit theory[1]として知られており，first hitとしてインスリン抵抗性による肝の脂肪蓄積（脂肪肝）があり，second hitとして酸化ストレス，エンドトキシン，TNFαなどの炎症性サイトカイン[2]などの要因が加わってNASHが発症すると考えられています。

▶NASHは進行性とされており，脂肪性肝炎から肝硬変症，一部肝癌を発症する場合もあります。また日本ではNASHによる肝硬変症は全肝硬変症の2％くらいとされています。原発性胆汁性肝硬変症と同じくらいです[3]。しかし，今後増加が予想されます。現在その背景に基づく様々な治療戦略が考えられています（図1）。

▶脂肪肝のどれくらいが脂肪性肝炎であるかについてはあまりよくわかっていませんが非アルコール性脂肪性肝疾患（non-alcoholic fatty liver disease：NAFLD）の約10％がNASHとされています。NASHの5～20％が5年から10年で肝硬変症に進行するとされています。

▶NASHとNAFLDの血液検査上の鑑別は不可能で，肝生検を必要とします。

2 脂肪肝はメタボリックドミノの上流

▶脂肪肝はメタボリックシンドロームの肝臓での表現型とされており，メタボリックドミノの上流と考えられます（図2）。そのため生活習慣病やメタボリックシンドロームの予防を考える上で，肝臓での変化を理解することは重要と考えられます。

▶食餌性肥満ラットでは，まず肝臓や内臓に脂肪が蓄積し，その後血液中のインスリン抵抗性が出現するという報告[4]があります。まず肝臓の脂肪蓄積を防ぐことが肥満症においてメタボリック症候群を防ぐための重要なstepになると考えられます。

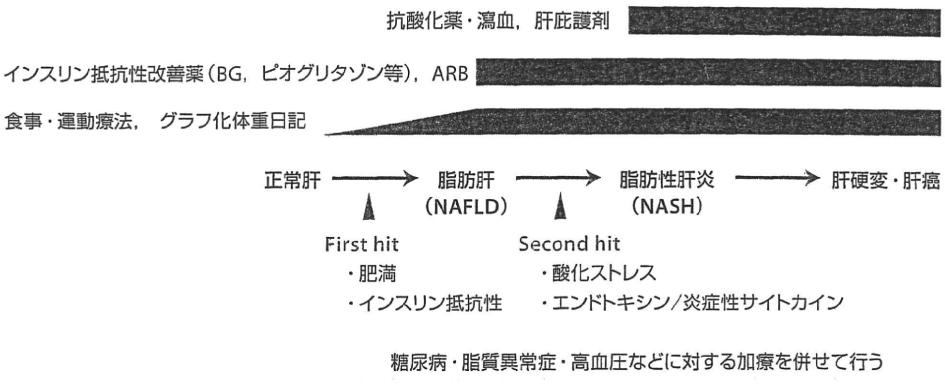

図1　一般的なNAFLD・NASHの病態と治療の考え方

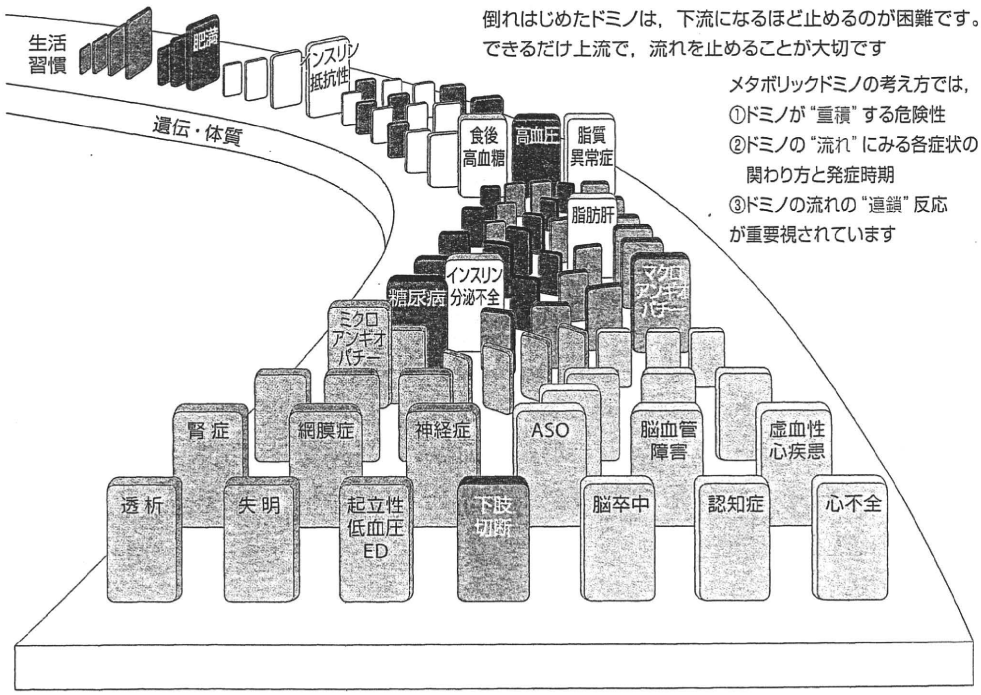

図2　メタボリックドミノ

（慶應義塾大学医学部内科伊藤裕教授原図）

▶実際，脂肪肝の患者では心血管イベントが多いということが知られています。当科においても400人の糖尿病合併脂肪肝の患者は5年間で心血管イベントが明らかに多いという結果でした[5]。このため，生活習慣病予防の観点から脂肪肝の治療が必要となります。

3 脂肪肝の診断

▶脂肪性肝疾患は肝細胞に中性脂肪が沈着して肝障害をきたす疾患の総称です。脂肪滴を伴う肝細胞が30％以上認められる場合，一般に脂肪肝と言います。

▶腹部超音波診断装置や腹部CT診断装置，MRIの診断能力の向上で，数％の脂肪沈着でも脂肪浸潤として認識されます。特に腹部超音波は簡便で，bright liver，liver-kidney contrast（肝・腎コントラスト），deep attenuation（深部減衰），blurring（血管の不明瞭化）があれば診断は容易です。

4 脂肪肝と脂肪性肝炎（NASH）の鑑別

▶NASHの統一診断基準はありませんが，以下の目安が多く用いられています。

①非飲酒者である（エタノール換算でアルコール摂取量が，男性≦30g/日，女性≦20g/日）

②画像診断で脂肪肝を認める

③病理組織像でsteatohepatitis（脂肪性肝炎）を認める

④肝炎ウイルスや血清学的な肝障害を認めない（確定診断のためには組織学的検査が必要）

▶現在，血清学的に鑑別はできません。肝生検を行い病理組織検査によるほかありません。非飲酒者という定義はあいまいで今後再検討の余地があります。

▶病理組織の診断基準はBluntの報告に基づくもので，線維化を主体としたstaging，炎症を主体としたgradingによる分類が用いられています。

▶最近ではscoringによる評価も試みられています。線維化マーカー（ヒアルロン酸やタイプⅣコラーゲン）が高い場合は線維化が示唆されますので有用とされています。AST/ALTの比が高い場合や血小板値が低値の場合は進行している病態であることが示唆されます。

▶画像診断で肝硬変症の場合はAFPやPIVKA Ⅱなどの腫瘍マーカーの測定も行い肝癌発症に対する注意も必要です。

5 脂肪肝の治療

(1) 体重制御が基本――でも難しい

▶いかに持続させるかが大事です。

▶減量により肝機能障害は速やかに改善します。数％の減量で肝機能は良好となり，脂肪肝は改善します。もし減量しても肝機能の改善がない場合は他の肝疾患を考えます。特に健康食品や

薬剤性の肝障害は考慮する必要があります。

▶実際，体重の減量が得られ，NAFLDの肝組織所見の改善が得られたとの報告[6]や栄養指導の短期的な介入が有効であったとの報告[7]がみられ，体重の制御がNAFLDの治療の中心であることが確認されています。

▶当科では行動療法としての「グラフ化体重日記」を中心に治療を行っています(☞07章/*p38*～)。しかし，体重の減量過程で挫折する患者も多く，適切な減量については課題が残されています。減量の実行と継続の困難さに加え，減量に伴う代謝の変化がその一因とも考えられています。

▶減量を長期(数年ではなく10年単位で)に維持するための戦略が必要と考えられています。

▶減量治療の実際については，04章(☞*p15*～)，05章(☞*p21*～)を参照して下さい。

▶減量の目安は標準体重の＋10％以内にし，1～2kg/月の速度で行うこと，運動療法も急激に行うことは避けることが重要です。

(2)薬物療法の留意点

▶脂肪肝を伴う肥満症の治療は，食事や運動による減量が原則です。食事療法や運動療法だけでは目標とする改善が得られない症例に，減量を目的にした薬剤投与を考慮します。

▶脂肪肝およびNASHはインスリン抵抗性を基盤に発症することが予想されており，インスリン抵抗性改善薬が注目されています。

▶薬物療法で改善しない高度肥満を伴った場合は外科的療法が考えられています。しかしいずれにしても減量が中心となります。

▶脂肪肝の病態を考え加療する必要があります。インスリン抵抗性改善薬，抗酸化薬，脂質異常症治療薬，肝庇護剤，その他ARBなどの薬物療法が報告されています(図1)。しかし多くは短期間での評価が多く，長期の評価が少ないことが今後の課題です。

6 治療目標：ALT値が正常の脂肪肝に対して治療が必要か

▶脂肪肝と診断したら，ALT値をみてみましょう。

▶ただし，ALTの正常値については論議があります。現在は基準値内としているALT値の中には脂肪肝の患者さんがたくさん含まれます。

▶ALT値が正常の脂肪肝に対して治療が必要か？――これは今後の検討ですが，最近のいろいろな報告をみてみますとALT値は30IU/L以下であることが重要です。当科の検討でも糖尿病合併脂肪肝の患者ではALT値が30IU/L以下の場合，心血管イベントが少ないということが明らかになりました。脂肪肝の管理でALT値30IU/L以下をめざすということが大事でしょう。

▶今後，肥満症においては，末梢代謝中心臓器である肝臓の病態解明はとても重要です。飽食の時代の現在，肝臓は余裕のある沈黙の臓器から，余裕のない(予備能のなくなった)臓器となっています。肝臓に少し余裕を残しながら生活するというのがこれからの生活習慣病に対する

基本的な接し方となります。肝臓に少し余裕を残すというのがALT値30 IU/L以下と考えてよいと思います。

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✍清家正隆，吉松博信

Influence of *ITPA* Polymorphisms on Decreases of Hemoglobin During Treatment with Pegylated Interferon, Ribavirin, and Telaprevir

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Polymorphisms of the inosine triphosphatase (*ITPA*) gene influence anemia during pegylated interferon (PEG-IFN) and ribavirin (RBV) therapy, but their effects during triple therapy with PEG-IFN, RBV, and telaprevir are not known. Triple therapy for 12 weeks, followed by PEG-IFN and RBV for 12 weeks, was given to 49 patients with RBV-sensitive (CC at rs1127354) and 12 with RBV-resistant (CA/AA) *ITPA* genotypes who had been infected with hepatitis C virus (HCV) of genotype 1. Decreases in hemoglobin levels were greater in patients with CC than CA/AA genotypes at week 2 (−1.63 ± 0.92 vs. −0.48 ± 0.75 g/dL, $P = 0.001$) and week 4 (−3.5 ± 1.1 vs. −2.2 ± 0.96, $P = 0.001$), as well as at the end of treatment (−2.9 ± 1.1 vs. −2.0 ± 0.86, $P = 0.013$). Risk factors for hemoglobin <11.0 g/dL at week 4 were female gender, age >50 years, body mass index (BMI) <23, and CC at rs1127354 by multivariate analysis. RBV dose during the first 12 weeks was smaller in patients with CC than CA/AA genotypes (52 ± 14% vs. 65 ± 21% of the target dose, $P = 0.039$), but the total RBV dose was no different between them (49 ± 17% and 54 ± 18% of the target, $P = 0.531$). Sustained virological response (SVR) was achieved in 70% and 64% of them, respectively ($P = 0.724$). *Conclusion: ITPA* polymorphism influences hemoglobin levels during triple therapy, particularly during the first 12 weeks while telaprevir is given. With careful monitoring of anemia and prompt adjustment of RBV dose, SVR can be achieved comparably frequently between patients with CC and CA/AA genotypes. (HEPATOLOGY 2011;53:415-421)

Abbreviations: BMI, body mass index; GWAS, genome-wide association study; HCV, hepatitis C virus; IFN, interferon; IL28B, *interleukin 28B;* ITPA, *inosine triphosphatase; PEG-IFN, pegylated interferon; RBV, ribavirin; SNP, single nucleotide polymorphism; SVR, sustained virological response.*

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Potential conflict of interest: Nothing to report.

Worldwide, 123 million people are estimated to have been infected with hepatitis C virus (HCV),[1] and ≈30% of them develop fatal liver disease such as cirrhosis and hepatocellular carcinoma.[2,3] Currently, the standard of care therapy for patients infected with HCV is pegylated interferon (PEG-IFN) and ribavirin (RBV) for 48 weeks.[4-6] However, the combined treatment can induce a sustained virological response (SVR), judged by the loss of detectable HCV RNA from serum 24 weeks after treatment completion, in at most 50% of patients infected with HCV-1, the genotype most prevalent and least responsive to IFN-based therapies.

Recently, Fellay et al.[7] reported that polymorphisms of the inosine triphosphatase (*ITPA*) gene in chromosome 20 (20p13) influence RBV-induced anemia in a genome-wide association study (GWAS). Single nucleotide polymorphism (SNP) at rs1127354 for proline-to-threonine substitution (P32T) in the second of eight

exons in the *ITPA* gene, as well as that at rs7270101 in the second intron, affects the expression of ITPA.[8-11] Patients infected with HCV-1 carrying the CC genotype at rs1127354 are more prone to develop anemia than those with CA/AA genotypes during the combination therapy, and the decrease in hemoglobin is greater in patients with the AA than AC/CC genotypes at rs7270101.[7] Their observations have been extended to many patients in a large-scale trial with pegIFN-α-2a on Caucasian and African Americans,[12] as well as in the Japanese receiving PEG-IFN-α-2b and RBV who were infected with HCV-1.[13]

For improving SVR in HCV-1 patients, protease inhibitors have been added to the standard treatment with PEG-IFN and RBV, and increased SVR by ≈20%.[14-16] However, such a gain in efficacy is not without trade-offs, represented by aggravation of anemia. Early decreases in hemoglobin levels during the triple therapy reach 4 g/dL, and they exceed ≈3.0 g/dL in the standard treatment.[14,15] Because there have been no reports focusing on the influence of *ITPA* genotypes on anemia developing in patients during triple therapy, hemoglobin levels were followed in 61 Japanese patients with HCV-1 who had received it. The results were correlated with polymorphisms at rs1127354 in the *ITPA* gene because the Japanese are monoallelic at rs7270101 and have the AA genotype exclusively.[11]

Patients and Methods

Study Cohort. This retrospective cohort study was performed in 61 patients with chronic hepatitis C who met the following inclusion and exclusion criteria. Inclusion criteria were: (1) diagnosed with chronic hepatitis C; (2) HCV-1 confirmed by sequence analysis in the NS5B region; (3) HCV RNA levels ≥5.0 log IU/mL determined by the COBAS TaqMan HCV test (Roche Diagnostics K.K. Tokyo, Japan); (4) Japanese aged from 20 to 65 years at the entry; and (5) body weight between ≥40 kg and ≤120 kg at the time of registration. Exclusion criteria were: (1) decompensated liver cirrhosis; (2) hepatitis B surface antigen in serum; (3) hepatocellular carcinoma or its history; (4) autoimmune hepatitis, alcoholic liver disease, hemochromatosis, or chronic liver disease other than chronic hepatitis C; (5) chronic renal disease or creatinine clearance ≤50 mL/min at the baseline; (6) hemoglobin ≤12 g/dL, neutrophil ≤1,500/mm^3 or platelet ≤100,000/mm^3 at baseline.

Of the 61 patients, 44 (72%) had received IFN-based treatment before. Relapse occurred in 29 (47%) and the remaining 15 (25%) did not respond (null-responders). All patients gave consent for analysis of SNPs in *ITPA* and interleukin 28 (*IL28B*) genes. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of Toranomon Hospital. Written informed consent was obtained from each patient.

Triple Treatment with PEG-IFN-α-2b, RBV, and Telaprevir. Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan), 750 mg, was administered 3 times a day at an 8-hour (q8) interval after each meal. Pegylated-IFN-α-2b (PEG-Intron, Schering Plough, Kenilworth, NJ) was injected subcutaneously at a median dose of 1.5 μg/kg (range: 1.32-1.71 μg/kg) once a week. RBV (Rebetol, Schering Plough) 200-600 mg was administered after breakfast and dinner. The RBV dose was adjusted by body weight: 600 mg for ≤60 kg; 800 mg for >60 kg ≈≤80 kg; and 1,000 mg for ≥80 kg. The triple therapy with PEG-IFN-α-2b, RBV, and telaprevir was continued for 12 weeks, and then switched to PEG-IFN-α-2b and RBV for an additional 12 weeks. It was withdrawn when hemoglobin levels decreased <8.5 g/dL. After the therapy was completed or discontinued, patients were followed for 24 weeks for SVR.

The RBV dose was cut by 200 mg in patients receiving 600 or 800 mg (by 400 mg in those receiving 1,000 mg) when hemoglobin decreased <12 g/dL, and by another 200 mg when it was below <10 g/dL. In addition, RBV was reduced by 200 mg in patients with hemoglobin <13 g/dL at baseline and those in whom it decreased by 1 g/dL to <13 g/dL within a week. PEG-IFN dose was reduced by one-half when the leukocyte count decreased <1,500/mm^3, neutrophil count <750/mm^3, or platelet count <80 × 10^3/mm^3; PEG-IFN was withdrawn when they decreased <1,000/mm^3, 500/mm^3, or 50 × 10^3/mm^3, respectively.

The triple therapy was withdrawn or stopped temporarily when hemoglobin decreased <8.5 g/dL. In patients in whom hemoglobin increased ≥8.5 g/dL within 2 weeks after the withdrawal, treatment was resumed with PEG-IFN and RBV 200 mg. A reduction of telaprevir (MP-424) dose was not permitted. It was discontinued when severe side effects appeared, whereas PEG-IFN and RBV were continued. Growth factors were not used for elevating hemoglobin levels.

Determination of ITPA Genotypes. *ITPA* (rs1127354) and *IL28B* (rs8099917 and rs12979860) were genotyped by the Invader assay, TaqMan assay, or direct sequencing, as described.[17,18]

Statistical Analyses. Continuous variables between groups were compared by the Mann-Whitney test (*U* test), and discontinuous variables by the chi-square test

Table 1. Baseline Characteristics of the 61 Patients Infected with HCV-1 Who Received Triple Therapy with Pegylated-Interferon, Ribavirin, and Telaprevir

	Total	*ITPA* Genotypes at rs1127354	
		CC	CA + AA
Demographic data			
Number	61	49	12
Sex (male/female)	34/27	28/21	6/6
Age (years)	56 (23-65)	55 (23-65)	58 (28-62)
Body weight (kg)	61.5 (41.0-92.9)	61.5 (41.0-92.9)	62.1 (44.4-81.1)
Body mass index (kg/m^2)	22.6 (17.6-32.4)	22.2 (17.6-32.4)	22.9 (17.8-26.5)
Genotypes of the *IL28B* gene			
rs8099917 (for 59 patients)			
(TT/TG + GG)	33/26	27/21	6/7
rs12979860 (for 57 patients)			
(CC/CT + TT)	30/27	36/22	4/5
Laboratory data			
Hemoglobin (g/dL)	14.4 (12.5-16.6)	14.4 (12.5-16.6)	14.2 (12.8-16.3)
Platelets (x 10^4/mm^3)	17.8 (9.1-33.8)	17.7 (9.1-33.8)	19.5 (13.1-31.6)
Albumin (g/dL)	3.9 (3.2-4.6)	3.9 (3.2-4.6)	3.9 (3.5-4.1)
Alanine aminotransferase (U/L)	39 (12-175)	41 (12-175)	28 (17-57)
Aspartate aminotransferase (U/L)	32 (15-137)	35 (15-137)	28 (20-35)
HCV RNA (log IU/mL)	6.7 (5.1-7.6)	6.8 (5.7-7.6)	6.6 (5.1-7.5)
HCV genotype 1a/1b	1/60	1/48	0/12
Previous IFN-based treatment			
Treatment naïve	17	12 (24%)	5 (42%)
Relapsed	29	23 (47%)	6 (50%)
Null response	15	14 (29%)	1 (8%)

Data are median values (range) or n.

and Fisher's exact test. Kaplan-Meier analysis and the log-rank test were applied to estimate and compare decreases of RBV dose between groups. Factors evaluated for influence on hemoglobin decrease by univariate analysis were: sex; age; body mass index (BMI); body weight; hemoglobin levels; initial PEG-IFN and RBV doses; amino acid substitutions in the HCV core protein; number of amino acid substitutions in the interferon sensitivity determining region; and *IL28B* polymorphisms (at rs8099917 and rs12979860). Factors associated with a decrease in hemoglobin levels ($P < 0.10$) were assessed by multiple logistic regression analysis, and the odds ratio (OR) with 95% confidence interval (CI) was determined. All analyses were performed using SPSS software (SPSS II v. 11.0, Chicago, IL), and a P-value < 0.05 was considered significant.

Results

Triple Therapy in Patients with HCV-1 Infection. Baseline characteristics of the 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 in the *ITPA* gene are compared in Table 1. They all were infected with HCV-1. There were no significant differences between them, except that alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were higher in patients with CC than CA/AA genotypes ($P = 0.041$ and $P = 0.008$, respectively). Overall, *IL28B* genotypes resistant to PEG-IFN and RBV, TT/TG at rs8099917, and CC/CT at rs12979860 were rather frequent, and possessed by 44% and 47%, respectively, of the patients. This was due to inclusion of 15 nonresponders to previous IFN-based therapies, corresponding to 25% of the 61 patients studied, most of whom (14/15 [93%]) possessed IFN-resistant genotypes (TT/TG and CC/CT). Six of them had low hemoglobin levels (<13 g/dL) at baseline and were started with an RBV dose decreased by 200 mg; they included five with CC and one with CA genotypes of the *ITPA* gene.

Modification of RBV Dose During Triple Therapy. RBV dose was reduced by ≥200 mg in all 61 patients studied during triple therapy because hemoglobin had decreased <12.0 g/dL in them. During the first 12 weeks of therapy while telaprevir was given, the proportion of patients receiving the full RBV dose differed between those with CC and CA/AA genotypes (Fig. 1). RBV dose reduction was started earlier in the 49 patients with CC than the 12 with CA/AA genotypes (2.6 ± 1.3 vs. 4.8 ± 3.1 weeks after the start, respectively, $P = 0.010$). Thus, during the first 12 weeks with telaprevir the RBV dose was smaller in patients with CC than CA/AA genotypes (52 ± 14% vs. 65 ± 21% of the target dose, $P = 0.039$). During the next 12

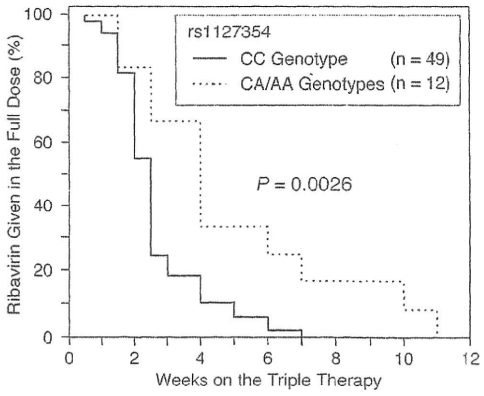

Fig. 1. Patients who received the full ribavirin dose during 12 weeks on triple therapy. The 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 are compared.

weeks without telaprevir, in contrast, the RBV dose was somewhat larger in patients with CC than CA/AA genotypes (47 ± 24% vs. 43 ± 20%, $P = 0.649$). The total RBV dose during 24 weeks on therapy was comparable between the 49 patients with CC and the 12 with CA/AA genotypes (49 ± 17% vs. 54 ± 18%, $P = 0.531$). In patients with the CC genotype, the RBV dose was no different between those who achieved SVR and those who did not (50 ± 18% vs. 47 ± 13%, $P = 0.728$). The RBV dose did not differ either in patients with CA/AA genotypes with and without SVR (57 ± 17% vs. 48 ± 20%, $P = 0.368$).

The total dose of PEG-IFN was comparable among 49 patients with CC and 12 with CA/AA genotypes (87 ± 23% vs. 86 ± 20% of the target, $P = 0.488$). The total telaprevir dose was no different either between them (87 ± 27% vs. 71 ± 36% of the target, $P = 0.098$). Telaprevir was discontinued in 10 of the 49 (20%) patients with CC and 5 of the 12 (42%) with CA/AA genotypes ($P = 0.147$).

Decreases in Hemoglobin Levels During Triple Therapy. Figure 2 compares decreases in hemoglobin levels between 49 patients with CC and 12 with CA/AA genotypes of the *ITPA* gene. Data of six patients were omitted because the triple therapy was withdrawn 4-10 weeks after the start, including five with CC and one with CA genotype. Hemoglobin decreased more in patients with CC than CA/AA genotypes at week 2 (−1.63 ± 0.92 vs. −0.48 ± 0.75 g/dL, $P = 0.001$) and week 4 (−3.5 ± 1.1 vs. −2.2 ± 0.96, $P = 0.001$). During week 8 through 12, hemoglobin reached the nadir of approximately −4 g/dL both in patients with CC and CA/AA genotypes. Thereafter, differences in hemoglobin decrease started to widen between patients with CC and CA/AA genotypes and were significant at week 20 (−3.0 ± 1.2 vs. −2.4 ± 0.88 g/dL, $P = 0.048$) and week 24 (−2.9 ± 1.1 vs. −2.0 ± 0.85 g/dL, $P = 0.013$).

SVR was achieved by 35 (71%) of the 49 patients with CC and 8 (67%) of the 12 with CA/AA genotypes ($P = 0.736$). Hemoglobin levels did not differ between them 24 weeks after the completion of triple therapy (−0.57 ± 1.1 vs. −0.17 ± 0.87 g/dL, $P = 0.271$). Of the 32 patients with TT genotype of the *IL28B* gene at rs8099917, 30 (94%) gained SVR, more frequently than 10 of the 26 (38%) with TG/GG genotypes ($P < 0.001$). Likewise, 29 of the 30 (97%) patients with CC genotype at rs12979860 achieved SVR, more frequently than 11 of the 27 (41%) with CT/TT genotypes ($P < 0.001$).

Factors Influencing Decreases in Hemoglobin Levels. Hemoglobin decreased <11 g/dL at week 4 during the triple therapy in 27 of the 61 (44%) patients. Factors for hemoglobin <11.0 g/dL were female gender, age >50 years, body weight <60 kg, BMI <23, and baseline hemoglobin <15 g/dL, as well as the CC genotype of the *ITPA* gene, in the univariate analysis (Table 2). Of them, female gender, age >50 years, BMI <23, and the CC genotype remained significant in the multivariate analysis. Hemoglobin levels lowered <8.5 g/dL during the triple therapy in 13 of the 61 (21%) patients. Factors for hemoglobin <8.5 g/dL were female gender, age >60 years, body weight <60 kg, BMI <23, and baseline hemoglobin <14 g/dL in the univariate analysis (Table 3). Of

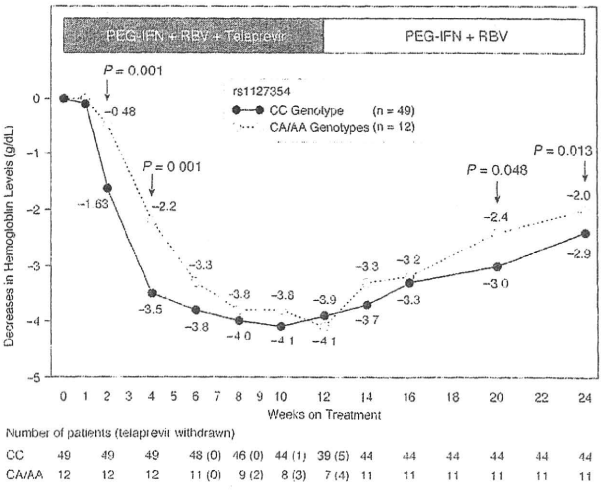

Fig. 2. Decreases in hemoglobin levels during triple therapy with telaprevir, PEG-IFN, and RBV. The 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 are compared. Patients evaluated at each timepoint are indicated below, with the number of patients in whom telaprevir was withdrawn (PEG-IFN and RBV continued) in parentheses.

Table 2. Univariate and Multivariate Analyses of Host and Viral Factors Associated with Low Hemoglobin Levels (< 11.0 g/dL) at Week 4 of Triple Therapy

Parameter	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	P	OR (95% CI)	P
Sex (female)	14.3 (4.1-50.0)	< 0.001	29.41 (3.8-250.0)	0.001
Age (> 50 years)	4.3 (1.0-17.5)	0.030	7.3 (1.1-47.6)	0.039
Body weight (< 60 kg)	11.5 (3.4-38.2)	< 0.001		
Body mass index (< 23)	8.4 (2.6-27.1)	< 0.001	17.2 (2.6-112.0)	0.003
Hemoglobin (< 15g/dL)	14.2 (3.5-57.4)	< 0.001		
ITPA gene (CC genotype)		0.062	36.8 (2.5-550.2)	0.009

Abbreviations: OR, odds ratio; CI, confidence level.

them, only age and body weight remained significant in the multivariate analysis.

Discussion

Anemia is a substantial risk in the standard of care therapy with PEG-IFN and RBV.[4-6] Triphosphorylated RBV accumulates in erythrocytes of patients who receive RBV, increasingly with RBV dose and duration, and causes oxidative damage to erythrocyte membranes toward extravascular hemolysis by the reticuloendothelial system.[19,20] Inosine triphosphate accumulates also in erythrocytes of individuals who have mutations in the *ITPA* gene, and results in benign red-cell enzymopathy.[8] The expression of *ITPA* is genetically controlled and reduced in individuals who have point mutations in the *ITPA* gene.[8-11] As another achievement of GWAS in hepatology,[21] in the wake of polymorphisms of the *IL28B* gene that influence the response to PEG-IFN and RBV,[22-24] polymorphisms in the *ITPA* gene has been reported to influence anemia caused by RBV.[7] How inosine triphosphate protects erythrocytes from hemolysis caused by RBV needs to be sorted out by *in vivo* and *in vitro* experiments. Inosine triphosphate may prohibit the accumulation of RBV in erythrocytes, or rather, it might act directly toward prohibition of hemolysis.

In the present study, 61 patients infected with HCV-1 received triple therapy with PEG-IFN, RBV, and telaprevir in the first 12 weeks followed by PEG-IFN and RBV in the second 12 weeks. Then the RBV dose and hemoglobin were compared between patients with CC and CA/AA genotypes in the *ITPA* gene. Two polymorphisms in the *ITPA* gene, in close linkage disequilibrium with an r^2 value of 0.65,[7] have been recognized in Caucasians (rs1127354 and rs7270107); the respective CA/AA and AC/CC genotypes decrease the activity of inosine triphosphatase and protect against anemia induced by RBV.[7,12] Because the Japanese are monoallelic at rs7270107 and possess the AA genotype exclusively,[11,25] only polymorphisms at rs1127354 were examined.

Of the 61 patients, 49 possessed the RBV-sensitive CC genotype and the remaining 12 had RBV-resistant CA/AA genotypes. Hemoglobin levels decreased both in patients with CC and CA/AA genotypes. They lowered ≈4 g/dL during weeks 8-12 on the triple therapy with telaprevir, and increased thereafter (Fig. 2). Between the two groups of patients, differences in hemoglobin decrease were greatest at week 4 (1.3 g/dL), as in the standard treatment with PEG-IFN and RBV.[7,12,13]

When anemia and other side effects occurred, doses of RBV, PEG-IFN, and telaprevir were modified. Of the 61 patients studied, 27 (44%) were women and most of them were in old age. Beyond 50 years of age, women are less responsive than men to the standard treatment with PEG-IFN and RBV, probably because estrogens with an antifibrotic potential decrease after menopause.[26] Stringent precautions had to be taken, therefore, by reducing the RBV dose in the patients in whom hemoglobin levels decreased <12 g/dL, rather than the conventional threshold of <10 g/dL.

Reductions of RBV dose due to anemia in patients who receive PEG-IFN and RBV are influenced by *ITPA* polymorphisms.[12] Also, in patients who had received the triple therapy the RBV dose had to be reduced more in

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Parameter	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	P	OR (95% CI)	P
Sex (female)	6.1 (1.5-25.1)	0.007		
Age (>60 years)	6.8 (1.8-26.0)	0.004	10.1 (1.9-53.9)	0.007
Body weight (<60 kg)	23.8 (2.9-200.0)	<0.001	33.3 (3.4-333.3)	0.003
Body mass index (<23)	14.1 (1.7-125.0)	0.001		
Hemoglobin (<14 g/dL)	4.3 (1.2-15.6)	0.023		

Abbreviations: OR, odds ratio; CI, confidence level.

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Body weight (<60 kg)	23.8 (2.9-200.0)	<0.001	33.3 (3.4-333.3)	0.003
Body mass index (<23)	14.1 (1.7-125.0)	0.001		
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patients with CC than CA/AA genotypes during the first 12 weeks while they received telaprevir (52 ± 14% vs. 65 ± 21% of the target dose, $P = 0.039$). During the second 12 weeks off telaprevir, the RBV dose was somewhat greater in patients with CC than CA/AA genotypes (47 ± 24% vs. 43 ± 20%, $P = 0.649$). Thus, the total RBV dose during 24 weeks of therapy was comparable between patients with CC and CA/AA genotypes (51 ± 15% and 57 ± 18%, $P = 0.724$). Likewise, the total dose of PEG-IFN (87 ± 23% vs. 86 ± 20% of the target, $P = 0.806$), as well as that of telaprevir (87 ± 27% vs. 71 ± 36% of the target, $P = 0.098$), was no different between patients with CC and CA/AA genotypes. SVR was achieved comparably frequently in them (71% vs. 67%, $P = 0.736$).

Decreases in hemoglobin levels during the first 12 week were similar between the current triple therapy cohort and previous patients receiving PEG-IFN and RBV.[12,13] The conservative hemoglobin levels chosen for RBV dose reduction may be a possible confounding factor on the impact of *ITPA* variants in anemia, which would have been greater should the RBV dose not be reduced in patients with RBV-sensitive CC genotypes.

ITPA polymorphisms at rs1127354 were associated with RBV-induced anemia in Japanese patients, without involvement of those at rs7270107 reported in Caucasian and African-American patients.[13] Thus, *ITPA* polymorphisms at rs1127354 would play a major role in protecting patients from RBV-induced anemia. CC/CA genotypes at rs1127354 occurs in 6% of the Caucasian population, much less often in the Oriental population, at 16%.[25,27] Although AC/CC genotypes at rs7270107 occurs in 13% of Caucasians, they do not exist in Orientals.[11,25] Obviously, different polymorphisms need to be examined in patients of distinct ethnicities when the influence on RBV-induced anemia is to be evaluated.

In confirmation of our previous report,[28] the triple therapy achieved SVR more frequently in patients with CC than CT/TT genotypes of *IL28* at rs12979860 (96% vs. 41%, $P < 0.001$). About two-thirds of studied patients accomplished SVR with the triple treatment, although one-fourth of them were nonresponders to previous IFN-based treatments; they are known to respond poorly to repeated treatments. This would lend further support to the efficacy of triple therapy being higher than treatment with pegylated IFN and RBV.

There are strong points in this study. First, *ITPA* polymorphisms influence RBV-induced anemia in the triple therapy. Second, polymorphisms at rs1127350, without involvement of those at rs7270107, protect against RBV-induced anemia. Third, the triple therapy can be applied with high efficacy by careful monitoring of hemoglobin and prompt modification of RBV dose. There are weak points in this study as well. First, it was a retrospective cohort study conducted in a small size of patients, especially those with CA/AA genotypes at rs1127350, and included null-responders to previous IFN-based therapies; the real impact of *ITPA* polymorphisms on RBV-induced anemia may have been obscured. Second, the study was conducted in Japanese patients, and the results may or may not be extended to patients of different ethnicities with distinct genetic backgrounds. Hopefully, the results presented herein will promote future studies in which the influence of the *ITPA* polymorphism on RBV-induced anemia will be pursued in larger scale and on patients of various ethnicities around the world.

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Amino Acid Substitution in HCV Core Region and Genetic Variation near the *IL28B* Gene Affect Viral Dynamics during Telaprevir, Peginterferon and Ribavirin Treatment

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Key Words
Hepatitis C virus · Core region · *IL28B* · Telaprevir · Peginterferon · Ribavirin · Viral dynamics

Abstract
Objectives: Genetic variation near the *IL28B* gene and substitution of aa 70 and 91 in the core region of HCV-1b are useful as predictors of treatment efficacy to telaprevir/pegylated interferon (PEG-IFN)/ribavirin, but its impact on viral dynamics is not clear. ***Methods:*** This study investigated predictive factors of viral dynamics during 12- or 24-week regimen of triple therapy in 80 Japanese adults infected with HCV-1b. ***Results:*** After 24 h of commencement of treatment, the proportion of patients with Arg70 and Leu91 substitutions in the core region who showed ≥3.0 log drop in HCV RNA level was significantly higher than that of patients with Gln70 (His70) and/or Met91. At 8 and 12 weeks, HCV RNA loss rate of patients with rs8099917 genotype TT near *IL28B* gene was significantly higher than that of patients with non-TT. Multivariate analysis identified substitution of aa 70 and 91 as a predictor of ≥3.0 log fall in HCV RNA level at 24 h (Arg70 and Leu91) and SVR (Arg70), and rs8099917 (TT) as a predictor of HCV RNA loss at 12 weeks and SVR. ***Conclusions:*** This study identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of viral dynamics during triple therapy.

Introduction

Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [1, 2]. At present, treatments based on interferon (IFN), in combination with ribavirin, are mainstay for combating HCV infection. In Japan, HCV genotype 1b (HCV-1b) in high viral loads (>100 kIU/ml) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis

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C [3]. Such a background calls for efficient treatments of Japanese patients with chronic HCV infection.

Even with pegylated IFN (PEG-IFN) combined with ribavirin, a sustained virological response lasting over 24 weeks after the withdrawal of treatment is achieved in at most 50% of the patients infected with HCV-1b and high viral loads [4, 5]. Recently, a new strategy was introduced in the treatment of chronic HCV infection by means of inhibiting protease in the NS3/NS4 of the HCV polyprotein. Of these, telaprevir (VX-950) was selected as a candidate agent for treatment of chronic HCV infection [6]. Later, it was found that telaprevir, when combined with PEG-IFN and ribavirin, gains a robust antiviral activity [7, 8]. Two previous studies (PROVE1 and PROVE2) showed that the 12- and 24-week regimen of telaprevir/PEG-IFN/ribavirin could achieve sustained virological response rates of 35–60 and 61–69% in patients infected with HCV-1, respectively [9, 10]. Furthermore, a recent study (PROVE3) also showed that the 24- and 48-week regimen of triple therapy could achieve sustained virological response rates of 51 and 53% in HCV-1 infected patients in whom initial PEG-IFN/ribavirin treatment failed, respectively [11].

Amino acid (aa) substitutions at positions 70 and/or 91 in the HCV core region of patients infected with HCV-1b and high viral loads are pretreatment predictors of poor virological response to PEG-IFN plus ribavirin combination therapy [12–14], and also affect clinical outcome, including hepatocarcinogenesis [15, 16]. Furthermore, genetic variations near the *IL28B* gene (rs8099917, rs12979860) on chromosome 19 as host-related factor, which encodes IFN-λ-3, are pretreatment predictors of virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals infected with HCV-1 [17–20], and also affect clinical outcome, including spontaneous clearance of HCV [21]. A recent report identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of sustained virological response to triple therapy of telaprevir/PEG-IFN/ribavirin in Japanese patients infected with HCV-1b [22]. However, it is not clear at this stage whether genetic variation near the *IL28B* gene and aa substitution of the core region can be used before therapy to predict viral dynamics during triple therapy.

The present study included 80 patients with HCV-1b and high viral loads, who received the triple therapy of telaprevir with PEG-IFN plus ribavirin. The aims of the study were to identify the pretreatment factors that could predict viral dynamics during treatment, including viral- (aa substitutions in the HCV core and NS5A regions) and host-related factors (genetic variation near *IL28B* gene).

Patients and Methods

Study Population

Between May 2008 and September 2009, 81 patients infected with HCV were recruited to this study at the Department of Hepatology in Toranomon Hospital in metropolitan Tokyo. 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 an informed consent before participating in this trial. Patients were divided into two groups: 20 (25%) patients were allocated to a 12-week regimen of triple therapy [telaprevir (MP-424), PEG-IFN and ribavirin] (the T12PR12 group), and 61 patients (75%) were assigned to a 24-week regimen of the same triple therapy for 12 weeks followed by dual therapy of PEG-IFN and ribavirin for 12 weeks (the T12PR24 group).

Eighty of the 81 patients met the following inclusion and exclusion criteria: (1) Diagnosis of chronic hepatitis C. (2) HCV-1b confirmed by sequence analysis. (3) HCV RNA levels of ≥5.0 log IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). (4) Japanese (Mongoloid) ethnicity. (5) Age at study entry of 20–65 years. (6) Body weight ≥35 kg and ≤120 kg at the time of registration. (7) Lack of decompensated liver cirrhosis. (8) Negativity for hepatitis B surface antigen (HBsAg) in serum. (9) Negative history of HCC. (10) No previous treatment for malignancy. (11) Negative history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C. (12) Negative history of depression, schizophrenia or suicide attempts, hemoglobinopathies, angina pectoris, cardiac insufficiency, myocardial infarction or severe arrhythmia, uncontrollable hypertension, chronic renal dysfunction or creatinine clearance of ≤50 ml/min at baseline, diabetes requiring treatment or fasting glucose level of ≥110 mg/dl, autoimmune disease, cerebrovascular disorders, thyroidal dysfunction uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication or anaphylaxis at baseline. (13) Hemoglobin level of ≥12 g/dl, neutrophil count ≥1,500/mm^3, and platelet count of ≥100,000/mm^3 at baseline. Pregnant or breast-feeding women or those willing to become pregnant during the study and men with a pregnant partner were excluded from the study. In this study, all of the 80 patients were evaluated for the pretreatment predictors for viral dynamics during triple therapy, and 77 of the 80 patients were followed up for at least 24 weeks after the completion of treatment. The treatment efficacy was evaluated by 24 weeks after the completion of therapy (sustained virological response), based on the COBAS TaqMan HCV test (Roche Diagnostics).

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered at 750 or 500 mg three times a day at an 8-hour (q8) interval after the meal. PEG-IFNα-2b (PEG-Intron; Schering Plough, Kenklworth, N.J., USA) was injected subcutaneously at a median dose of 1.5 μg/kg (range 1.3–2.0 μg/kg) once a week. Ribavirin (Rebetol; Schering Plough) was administered at 200–600 mg twice a day after breakfast and dinner (daily dose 600–1,000 mg).

PEG-IFN and ribavirin were discontinued or their doses reduced, as required, upon reduction of hemoglobin level, leukocyte count, neutrophil count or platelet count, or the development of adverse events. Thus, the dose of PEG-IFN was reduced by 50% when the leukocyte count decreased below 1,500/mm^3, neutro-

Table 1. Profile and laboratory data at commencement of telaprevir, peginterferon and ribavirin triple therapy in Japanese patients infected with HCV-1b

Demographic data	
Number of patients	80
Sex, M/F	43/37
Age, years*	55 (23–65)
History of blood transfusion	24 (20.0%)
Family history of liver disease	13 (16.3%)
Body mass index*	22.5 (13.2–32.4)
*Laboratory data**	
Level of viremia, log IU/ml	6.8 (5.1–7.6)
Serum aspartate aminotransferase, IU/l	34 (15–118)
Serum alanine aminotransferase, IU/l	42 (12–175)
Serum albumin, g/dl	3.9 (3.3–4.6)
Gamma-glutamyl transpeptidase, IU/l	36 (9–229)
Leukocyte count, per mm^3	4,800 (2,800–8,100)
Hemoglobin, g/dl	14.3 (11.7–16.8)
Platelet count, $\times 10^4/mm^3$	17.3 (9.5–33.8)
α-Fetoprotein, μg/l	4 (2–39)
Total cholesterol, mg/dl	180 (112–276)
Fasting plasma glucose, mg/dl	92 (64–125)
Treatment	
PEG-IFNα-2b dose, μg/kg*	1.5 (1.3–2.0)
Ribavirin dose, mg/kg*	11.5 (7.2–18.4)
Telaprevir dose, 1,500/2,250 mg/day	10/70
Treatment regimen (T12PR12 group/T12PR24 group)	20/60
Amino acid substitutions in the HCV-1b	
Core aa 70, arginine/glutamine (histidine)	47/33
Core aa 91, leucine/methionine	43/37
ISDR of NS5A, wild-type/non-wild-type	76/4
Genetic variation near IL28B *gene*	
rs8099917 genotype, TT/TG/GG/ND	46/30/2/2
rs12979860 genotype, CC/CT/TT/ND	43/31/2/4
Past history of IFN therapy	
Treatment naive	27
Relapsers to previous treatment	33
Nonresponders to previous treatment	20

Data are numbers and percentages of patients, except those denoted by *, which represent the median (range) values.
ND = Not determined.

phil count below 750/mm^3 or platelet count below 80,000/mm^3; PEG-IFN was discontinued when these counts decreased below 1,000/mm^3, 500/mm^3 or 50,000/mm^3, respectively. When hemoglobin decreased to <10 g/dl, the daily dose of ribavirin was reduced from 600 to 400, 800 to 600 and 1,000 to 600 mg, depending on the initial dose. Ribavirin was withdrawn when hemoglobin decreased to <8.5 g/dl. However, the dose of telaprevir (MP-424) remained the same, and its administration was stopped when the discontinuation was appropriate for the development of adverse events. In those patients who discontinued telaprevir, treatment with PEG-IFNα-2b and ribavirin was also terminated.

Table 1 summarizes the profiles and laboratory data of the 80 patients at the commencement of treatment. They included 43 males and 37 females, aged 23–65 years (median 55 years).

Measurement of HCV RNA

The antiviral effects of the triple therapy on HCV were assessed by measuring plasma HCV RNA levels. In this study, HCV RNA levels during treatment were evaluated at least once every month before, during, and after therapy. Furthermore, to investigate the pretreatment predictors for viral dynamics, HCV RNA levels during treatment were evaluated at 7 time points; 24 h, 1, 2, 4, 6, 8 and 12 weeks after the commencement of treatment. HCV RNA levels during treatment were evaluated in 80 (100%), 80 (100%), 80 (100%), 79 (98.8%), 75 (93.8%), 74 (92.5%), and 69 (86.3%) of the 80 patients, at the above time intervals, respectively. HCV RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/ml, and the undetectable samples were defined as loss of HCV RNA. Especially, falls in HCV RNA levels at 24 h relative to baseline were investigated as very early dynamics.

Detection of Amino Acid Substitutions in Core and NS5A Regions of HCV-1b

With the use of HCV-J (accession No. D90208) as a reference [23], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed on 80 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [12]. The sequence of 2209–2248 aa in the NS5A of HCV-1b (IFN sensitivity-determining region; ISDR) reported by Enomoto et al. [24] was determined, and the numbers of aa substitutions in ISDR were defined as wild-type (0, 1) or non-wild-type (≥2). In the present study, aa substitutions of the core region and NS5A-ISDR of HCV-1b were analyzed by direct sequencing [22].

Genetic Variation near IL28B Gene

Samples for genomewide association survey were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip. Genotyping data were subjected to quality control before the data analysis. Genotyping for replication and fine mapping was performed by use of the Invader assay, TaqMan assay, or direct sequencing as described previously [25, 26].

In this study, genetic variations near *IL28B* gene (rs8099917, rs12979860), reported as the pretreatment predictors of treatment efficacy and clinical outcome [17–22], were investigated.

Statistical Analysis

Nonparametric tests (χ^2 test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly contributed to viral dynamics and sustained virological response. The ORs and 95%CI were also calculated. All p values less than 0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($p < 0.05$) on univariate analysis were entered into

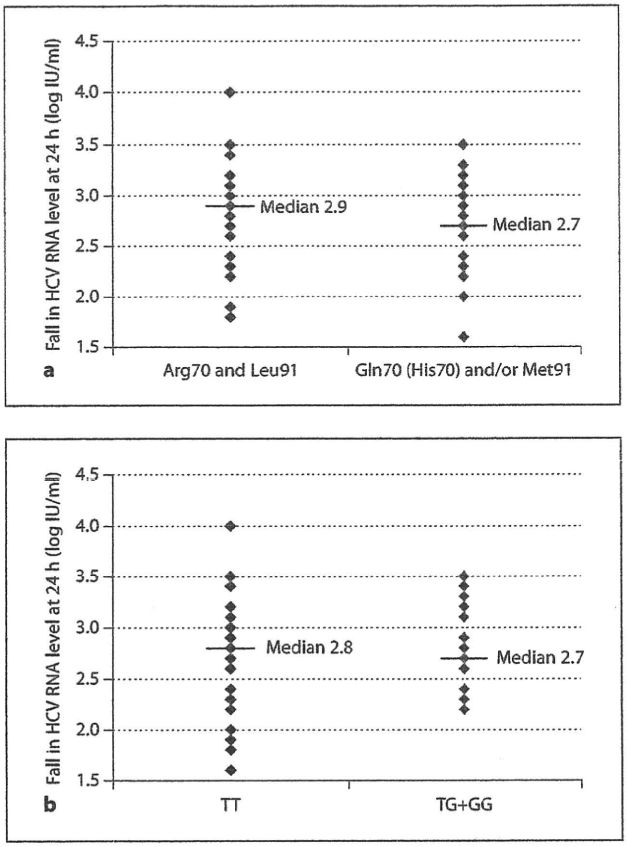

Fig. 1. a Very early dynamics according to amino acid substitutions in core region. After 24 h of commencement of the triple therapy, patients with Arg70 and Leu91 (median 2.9 log IU/ml; range 1.8–4.0 log IU/ml) significantly showed the steeper decline of HCV RNA level than those with Gln70 (His70) and/or Met91 (median 2.7 log IU/ml; range 1.6–3.5 log IU/ml). **b** Very early dynamics according to genetic variation near the *IL28B* gene. After 24 h of commencement of the triple therapy, the decline of HCV RNA level of patients with rs8099917 genotype TT (median 2.8 log IU/ml; range 1.6–4.0 log IU/ml) was not significantly different from that of patients with genotype TG and GG (median 2.7 log IU/ml; range 2.2–3.5 log IU/ml).

multiple logistic regression analysis to identify significant independent predictive factors. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. The potential pretreatment factors associated with treatment efficacy included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase (γGTP), leukocyte count, hemoglobin, platelet count, HCV RNA level, α-fetoprotein, total cholesterol, fasting blood sugar, PEG-IFN dose/body weight, ribavirin dose/body weight, telaprevir dose/day, treatment regimen of triple therapy, past history of IFN therapy, genetic variation near the *IL28B* gene, and amino acid substitution in the core region, and NS5A-ISDR. Statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, Ill., USA).

Results

Virological Response to Therapy and Loss of HCV RNA during Treatment

Sustained virological response was achieved by 63.6% (49 of 77 patients). The disappearance rate of HCV RNA during treatment was 0% (0 of 80), 1.3% (1 of 80), 33.8% (27 of 80), 81.0% (64 of 79), 90.7% (68 of 75), 94.6% (70 of 74), and 89.9% (62 of 69) at 24 hours, 1, 2, 4, 6, 8, and 12 weeks, respectively.

Very Early Dynamics according to Amino Acid Substitutions in Core Region and Genetic Variation near the IL28B Gene

After 24 h of commencement of the triple therapy, the proportion of patients with Arg70 and Leu91 substitutions who showed ≥3.0 log drop in HCV RNA level (45.2%; 14 of 31 patients) was significantly higher than that of patients with Gln70 (His70) and/or Met91 (14.3%; 7 of 49) ($p = 0.004$). Thus, patients with Arg70 and Leu91 (median 2.9 log IU/ml; range 1.8–4.0 log IU/ml) significantly showed the steeper decline of HCV RNA level than those with Gln70 (His70) and/or Met91 (median 2.7 log IU/ml; range 1.6–3.5 log IU/ml) (fig. 1a).

After 24 h of commencement of treatment, the proportion of patients with rs8099917 genotype TT who showed ≥3.0 log drop in HCV RNA level (30.4%; 14 of 46 patients) was not significantly different from that of patients with genotype TG and GG (21.9%; 7 of 32). Thus, the decline of HCV RNA level of patients with genotype TT (median 2.8 log IU/ml; range 1.6–4.0 log IU/ml) was not significantly different from that of patients with genotype TG and GG (median 2.7 log IU/ml; range 2.2–3.5 log IU/ml) (fig. 1b).

Hence, the fall in HCV RNA level at 24 h was influenced by aa substitution patterns in the core region, but was independent of genetic variation near *IL28B* gene.

Rates of Loss of HCV RNA according to Amino Acid Substitutions in Core Region and Genetic Variation near the IL28B Gene

According to the substitution of core aa 70 and 91, the rate of HCV RNA loss of patients with Arg70 and Leu91 was not significantly different from that of patients with