

資料4 HCV についての外注検査

IL28B SNP、HCV コア領域・NS5A 領域のアミノ酸変異は保険適用外であるものの、外注検査で測定可能である。各施設の検査会社担当者に直接照会されたい。

(1) IL28B SNP 測定

ある検査会社では、専用容器(EDTA-2Na 加)、検体量 5.0 ml、報告日数 12~16 日としている。なお、価格については各施設の検査会社担当者に直接照会されたい。

なお、IL28B 測定はヒトゲノムを検体としており、医療領域では「医療・介護関係事業者における個人情報の適切な取り扱いのためのガイドライン(厚生労働省)」、および「遺伝学的検査に関するガイドライン」(遺伝医学関連 10 学会)、「ファーマコゲノミクス検査の運用指針」(日本臨床検査医学会など)を、また研究領域では「ヒトゲノム・遺伝子解析研究に関する倫理指針」(文部科学省・厚生労働省・経済産業省)を遵守する必要がある。したがって、個人の遺伝情報の保護に十分留意しつつ、IL28B SNP 検査について患者に対して文書による説明を十分に行い、同意を得なければならない。検査会社によっては説明文書・同意書を用意しているところもあるので、参考にされたい。また、施設内に倫理委員会が設置されていれば、IL28B SNP 測定についてあらかじめ倫理委員会に申請し、承認を得るべきである。

(2) HCV コア領域・NS5A 領域のアミノ酸変異測定

ある検査会社によればそれぞれ以下のとおりである。

HCV コア領域 70 番・91 番アミノ酸変異:専用容器、検体量 5.0 ml、報告日数 10~14 日。

HCV NS5A 領域アミノ酸変異(ISDR):専用容器、検体量 5.0 ml、報告日数 10~14 日。

なお、価格については各施設の検査会社担当者に直接照会されたい。

参考資料 平成 23 年度厚生労働省科学研究費肝炎等克服緊急対策研究事業(肝炎分野) ウイルス肝炎における最新の治療法の標準化を目指す研究班による平成 24 年 B 型 C 型慢性肝炎・肝硬変治療ガイドライン

(http://www.jsh.or.jp/medical/date/H24_guideline.pdf)

Treatment Guidelines of Hepatitis C

The Committee for Hepatitis Clinical Guidelines, Japan Society of Hepatology

Key words: hepatitis C guidelines telaprevir interferon ribavirin

Kanzo 2012; 53: 355—395

The Committee for Hepatitis Clinical Guidelines, JSH

Yasuhiro Asahina¹, Namiki Izumi², Makoto Oketani³, Hiromitsu Kumada⁴, Kazuhiko Koike⁵,
Fumitaka Suzuki⁶, Hajime Takikawa^{6,*}, Atsushi Tanaka^{6,*}, Hirohito Tsubouchi³, Norio Hayashi⁷,
Naoki Hiramatsu⁸, Hiroshi Yotsuyanagi⁹ (In order of the Japanese syllabary)

- 1) Department of Gastroenterology and Hepatology, Department for Hepatitis Control, Tokyo Medical and Dental University, Tokyo, Japan
- 2) Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan
- 3) Digestive and Lifestyle Disease, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
- 4) Department of Hepatology, Toranomon Hospital, Tokyo, Japan
- 5) Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan (Director General of JSH)
- 6) Department of Medicine, Teikyo University School of Medicine, Tokyo, Japan
- 7) Kansai Rosai Hospital, Amagasaki, Japan
- 8) Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Osaka, Japan
- 9) Department of Internal Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

*Corresponding author; a-tanaka@med.teikyo-u.ac.jp

[†]Chair, The Committee for Hepatitis Clinical Guidelines

© 2012 The Japan Society of Hepatology

Original article

Age and total ribavirin dose are independent predictors of relapse after interferon therapy in chronic hepatitis C revealed by data mining analysis

Masayuki Kurosaki¹, Naoki Hiramatsu², Minoru Sakamoto³, Yoshiyuki Suzuki⁴, Manabu Iwasaki⁵, Akihiro Tamori⁶, Kentaro Matsuura⁷, Sei Kakinuma⁸, Fuminaka Sugauchi⁹, Naoya Sakamoto⁸, Mina Nakagawa⁸, Hiroshi Yatsuhashi¹⁰, Namiki Izumi^{1*}

¹Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan

²Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Osaka, Japan

³First Department of Internal Medicine, University of Yamanashi, Yamanashi, Japan

⁴Department of Hepatology, Toranomon Hospital, Tokyo, Japan

⁵Department of Computer and Information Science, Seikei University, Tokyo, Japan

⁶Department of Hepatology, Osaka City University Medical School, Osaka, Japan

⁷Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

⁸Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, Japan

⁹Department of Gastroenterology, Nagoya Koseiin Medical Welfare Center, Nagoya, Japan

¹⁰Clinical Research Center, National Nagasaki Medical Center, Nagasaki, Japan

*Corresponding author e-mail: nizumi@musashino.jrc.or.jp

Background: This study aimed to define factors associated with relapse among responders to pegylated interferon (PEG-IFN) plus ribavirin (RBV) therapy in chronic hepatitis C.

Methods: A cohort of genotype 1b chronic hepatitis C patients treated with PEG-IFN plus RBV and who had an undetectable HCV RNA by week 12 ($n=951$) were randomly assigned to model derivation ($n=636$) or internal validation ($n=315$) groups. An independent cohort ($n=598$) were used for an external validation. A decision tree model for relapse was explored using data mining analysis.

Results: The data mining analysis defined five subgroups of patients with variable rates of relapse ranging from 13% to 52%. The reproducibility of the model was confirmed by internal and external validations ($r^2=0.79$

and 0.83, respectively). Patients with undetectable HCV RNA at week 4 had the lowest risk of relapse (13%), followed by patients <60 years with undetectable HCV RNA at week 5–12 who received ≥ 3.0 g/kg of body weight of RBV (16%). Older patients with a total RBV dose <3.0 g/kg had the highest risk of relapse (52%). Higher RBV dose beyond 3.0 g/kg was associated with further decrease of relapse rate among patients <60 years (up to 11%) but not among older patients whose relapse rate remained stable around 30%.

Conclusions: Data mining analysis revealed that time to HCV RNA negativity, age and total RBV dose was associated with relapse. To prevent relapse, ≥ 3.0 g/kg of RBV should be administered. Higher dose of RBV may be beneficial in patients <60 years.

Introduction

The currently recommended therapy for chronic hepatitis C is a combination of pegylated interferon (PEG-IFN) plus ribavirin (RBV) [1]. This therapy is effective in 50% of patients with HCV genotype 1b [2,3]. The most reliable predictor of sustained virological response (SVR) is the response during early weeks of therapy. A satisfactory response to therapy in

the early weeks is associated with a high rate of SVR [4–8]. A basic concept of response-guided therapy is to modify the duration of therapy according to the time to HCV RNA negativity. Extended therapy may be given to patients with delayed virological response [9–13]. Modification of duration of therapy or drug dose may also be necessary in patients with early virological

response (EVR), because approximately 20% of these patients experience relapse after the completion of 48 weeks of therapy. Recent reports have revealed that single nucleotide polymorphisms located near the *IL28B* gene are strongly associated with SVR or a null response to PEG-IFN plus RBV therapy [14–16]. However, single nucleotide polymorphisms located near the *IL28B* gene are not associated with relapse after EVR [17]. Identification of risk factors for relapse among patients with virological response may lead to more individualized therapy and improved SVR rate.

Decision tree analysis, a core component of data mining analysis, is a method that explores data to develop predictive models [18]. This method has been originally used in business and recently in medical fields [19–25]. Decision tree analysis was successfully used to build a predictive model of EVR [26] and SVR to PEG-IFN plus RBV combination therapy in chronic hepatitis C [17,27,28]. The results of the analysis are presented as a tree structure, which is easy to understand and use in clinical practice. Patients can be allocated into

subgroups by simply following the flowchart form of the decision tree [29].

In the present study, we used decision tree analysis to identify predictors of relapse among patients who achieved EVR to PEG-IFN plus RBV therapy, and to define a more individualized therapeutic strategy beyond response-guided therapy.

Methods

Patients

This is a multicentre retrospective cohort study involving Musashino Red Cross Hospital, Toranomon Hospital, Tokyo Medical and Dental University, Osaka University, Nagoya City University, Yamanashi University, Osaka City University, and their related hospitals. The inclusion criteria were chronic hepatitis C patients treated with PEG-IFN- α 2b plus RBV, genotype 1b, pretreatment HCV RNA titre >100 KIU/ml as confirmed by quantitative PCR; Cobas Amplicor HCV Monitor version 2.0; Roche Diagnostic Systems, Pleasanton, CA, USA), an undetectable HCV RNA level within week 12 after the start of therapy, no coinfection with HBV or HIV, and no other causes of liver disease. Patients were treated with PEG-IFN- α 2b (1.5 μ g/kg) subcutaneously every week plus a daily weight-adjusted RBV dose (600 mg for patients weighing <60 kg, 800 mg for patients weighing 60–80 kg and 1,000 mg for patients weighing >80 kg). Dose reduction or discontinuation of PEG-IFN and RBV was considered based on the recommendations of the package inserts and the discretion of physicians at each university and hospital. The standard duration of therapy was set at 48 weeks, but extension of duration was allowed and implemented at the discretion of each physician. The duration of therapy was extended beyond 48 weeks in 118 patients (mean duration was 56.3 weeks, ranging from 49 to 72 weeks). Although the exact reason for the prolonged treatment in each case was not available, one reason may be that each physician tried to achieve high adherence of RBV by extending the duration of therapy. Another reason may be the late time point of HCV RNA negativity even within early virological response. Among 118 patients, time to HCV RNA negativity was between 9 to 12 weeks in 56% of patients.

A total of 951 patients fulfilled the study criteria. The baseline characteristics and representative laboratory test results are listed in Table 1. For analysis, patients were randomly assigned to either the model derivation (636 patients) or internal validation (315 patients) groups. There were no significant differences in the clinical backgrounds between these two groups. For external validation of the model, we collaborated with another multicentre study group consisting of 29 medical centres and hospitals belonging to the National

Table 1. Background of study population

Characteristic	Value
Age, years	54.9 (10.8)
Gender	–
Male, <i>n</i> (%)	557 (59)
Female, <i>n</i> (%)	394 (41)
Body mass index, kg/m ²	23.2 (3.3)
Albumin, g/dl	4.1 (1.8)
Creatinine, mg/dl	0.7 (0.2)
AST, IU/l	60.6 (46.2)
ALT, IU/l	80.7 (77.2)
GGT, IU/l	52.0 (60.0)
White blood cell count, cells/ μ l	4,993 (1,363)
Haemoglobin, g/dl	15.9 (52.6)
Platelets, 10 ⁹ /l	174.4 (6.1)
HCV RNA, KIU/ml	1,655 (1,455)
Fibrosis stage	–
F1–2, <i>n</i> (%)	626 (66)
F3–4, <i>n</i> (%)	98 (10)
NA, <i>n</i> (%)	227 (24)
Time to HCV RNA negativity 4/8/12 weeks	–
4 Weeks, <i>n</i> (%)	233 (24)
8 Weeks, <i>n</i> (%)	386 (41)
12 Weeks, <i>n</i> (%)	332 (35)
Treatment duration, weeks	42 (13)
Total RBV dose, g/kg body weight	3.1 (1.3)
Total PEG-IFN dose, μ g/kg body weight	62.5 (38.6)
Outcome	–
Relapse, <i>n</i> (%)	238 (25)
SVR, <i>n</i> (%)	713 (75)

Total *n*=951. Data are expressed as mean (sd) unless otherwise indicated. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ -glutamyltransferase; NA, not available; PEG-IFN, pegylated interferon; RBV, ribavirin; SVR: sustained virological response.

Hospital Organization (Japan). A dataset collected from 598 patients who were treated with PEG-IFN- α 2b plus RBV and had undetectable HCV RNA within week 12 were used for external validation. Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional review committees of all concerned hospitals.

Laboratory tests

Haematological tests, blood chemistry and HCV RNA titre were analysed before therapy and at least once every month during therapy. Rapid virological response (RVR) was defined as an undetectable HCV RNA level at week 4, and complete early virological response (cEVR) was defined as an undetectable HCV RNA level at week 5 through week 12 after the start of therapy. SVR was defined as an undetectable HCV RNA level 24 weeks after the completion of therapy. Detection of HCV RNA level was based on qualitative PCR with a lower detection limit of 50 IU/ml (Amplicor; Roche Diagnostic Systems). A database of pretreatment variables included haematological tests (haemoglobin level, white blood cell count and platelet count), blood chemistry tests (serum levels of creatinine, albumin, aspartate aminotransferase, alanine aminotransferase, γ -glutamyltransferase, total cholesterol, triglycerides and HCV RNA titre), stage of histological fibrosis and patient characteristics (age, sex and body mass index). Post-treatment variables included time to HCV RNA negativity, calculated total RBV dose (g/kg of body weight), and calculated total PEG-IFN dose (μ g/kg of body weight).

Statistical analysis

The Student's *t*-test was used for the univariable comparison of quantitative variables and Fisher's exact test was used for the comparison of qualitative variables. Logistic regression models with backward selection procedures were used for multivariable analysis of factors associated with relapse. IBM SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA) was used for analysis. For the decision tree analysis [30], the data mining software IBM SPSS Modeler 14 (SPSS Inc.) was used, as reported previously [17,26–28]. The decision tree analysis, the core component of the data mining, belongs to a family of non-parametric regression methods based on binary recursive partitioning of data. In this analysis, the software automatically explored the database to determine optimal split variables to build a decision tree structure. A statistical search algorithm evaluate the model derivation group to determine the optimum variables and cutoff values and to yield the most significant division of patients into two subgroups that were as homogeneous as possible for the probability

of relapse. Once patients were divided into 2 subgroups, the analysis was automatically repeated on each subgroup in the same way until either no additional significant variable was detected or the number of patients was <20. Finally all patients were classified into particular subgroups that are homogeneous with respect to the probabilities of relapse.

Results

The decision tree model for the prediction of relapse

The overall rate of relapse was 26% in the model derivation group. The decision tree analysis selected three variables that are associated with relapse: time to HCV RNA negativity, age and total RBV dose (Figure 1). Time to HCV RNA negativity was selected as the best predictor of relapse. The rate of relapse was 13% for patients with RVR compared to 30% for patients with cEVR. Among patients with cEVR, age was selected as the variable of second split. Patients <60 years had a lower probability of relapse (22%) compared with those \geq 60 years (41%). The total RBV dose was selected as the third variable of split with an optimal cutoff of 3.0 g/kg of body weight. The rate of relapse was lower in patients who received \geq 3.0 g/kg of body weight of RBV compared to patients who received <3.0 g/kg of body weight (among patients <60 years rates were 16% versus 32% and among patients \geq 60 years rates were 26% versus 52%, respectively).

According to this decision tree, the patients were divided into five groups with different rates of relapse ranging from 13% to 52%. Patients with RVR had the lowest risk of relapse. Among patients with cEVR, patients <60 years who received \geq 3.0 g/kg of body weight of RBV also had a low risk of relapse (16%). By contrast, patients who received <3.0 g/kg of body weight of RBV had higher than the average risk of relapse, especially in patients \geq 60 years (52%).

Validation of the decision tree model

The decision tree model was validated using an internal validation group that was not included in the model derivation. The rates of relapse for each subgroup of patients were correlated closely between the model derivation and the internal validation group ($r^2=0.79$; Figure 2A). When validated using an external validation group, the rates of relapse for each subgroup of patients were again correlated closely between the model derivation and the external validation group. ($r^2=0.83$; Figure 2B).

Multivariable logistic regression analysis for factors associated with relapse

Univariable and multivariable analysis was performed using the combined population of model derivation and internal validation group. Univariable analysis found

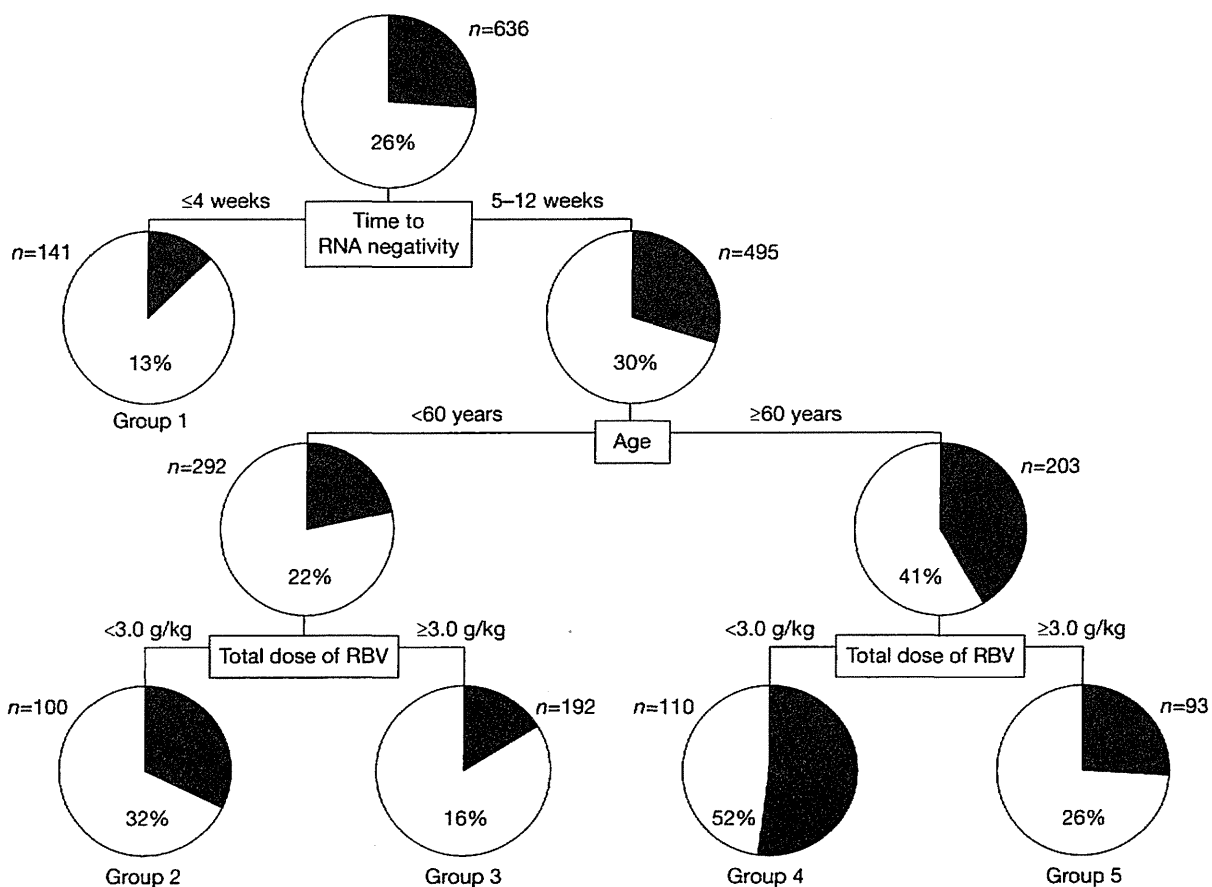
that age, sex, serum levels of creatinine, haemoglobin, platelet count, HCV RNA titre, time to HCV RNA negativity, total PEG-IFN dose and total RBV dose were associated with relapse. Duration of therapy was not associated with reduction in relapse rate. Multivariable analysis including these factors showed that age, total RBV dose, serum level of creatinine, and time to HCV RNA negativity were independent predictors of relapse (Table 2). Creatinine was not selected as a splitting variable in data mining analysis probably due to the limitation to stop the analysis when the number of patients was <20. Using the combined population of model derivation and internal validation group, patients in each subgroup of decision tree model were further stratified by creatinine levels and the effect of creatinine level on relapse was analysed. Among patients with RVR, the rate of relapse did not differ

between patients with creatinine levels of <0.7 g/dl and ≥ 0.7 g/dl and were 12% and 12%, respectively. Among patients with cEVR, the rate of relapse was higher in patients with creatinine levels of <0.7 g/dl compared to those with creatinine levels of ≥ 0.7 g/dl and were 39% versus 23%, respectively, for patients <60 years who received <3.0 g/kg of body weight of RBV, 19% versus 14% for patients <60 years who received ≥ 3.0 g/kg of body weight of RBV, 58% versus 41% for patients ≥ 60 years who received <3.0 g/kg of body weight of RBV, and 42% versus 26% for patients ≥ 60 years who received ≥ 3.0 g/kg of body weight of RBV.

Effect of age and total RBV dose on relapse among patients with cEVR

The effect of total RBV dose on relapse was analysed among patients with cEVR in a combined group of

Figure 1. The decision-tree model of relapse among patients with rapid virological response or complete early virological response



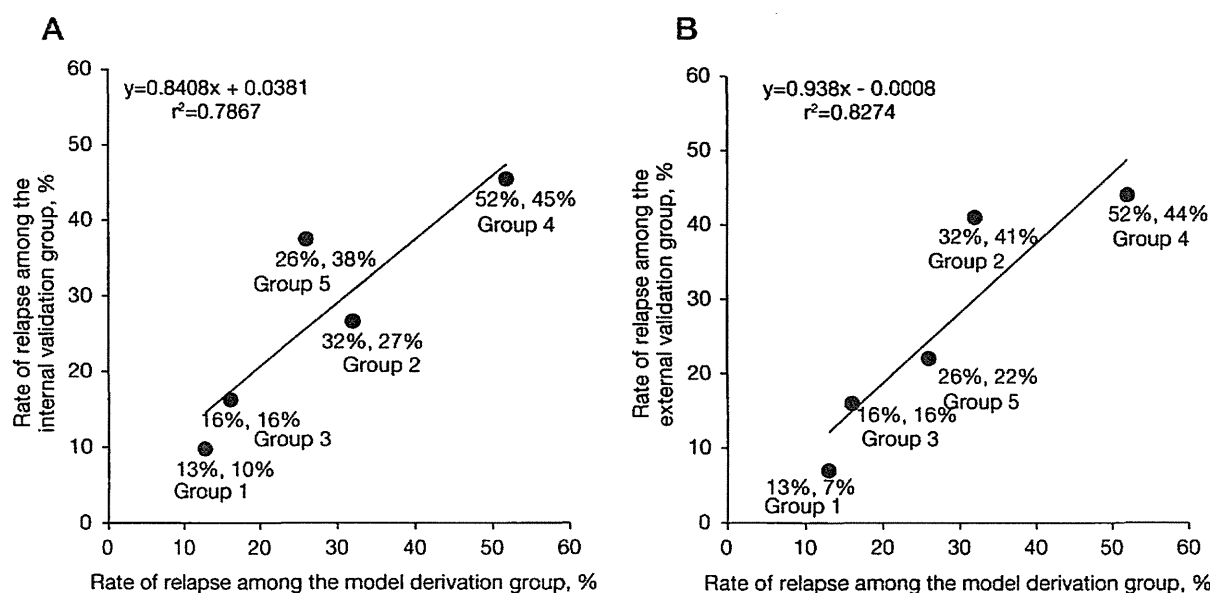
Boxes indicate the factors used for splitting and the cutoff values for the split. Pie charts indicate the rate of relapse for each group of patients after splitting. Terminal groups of patients discriminated by the analysis are numbered from 1 to 5. The rate of relapse was higher than average (>26%) in subgroups 2 and 4, where total ribavirin (RBV) dose was <3 g/kg of body weight.

model derivation and internal validation ($n=718$). The relapse rate decreased with an increase in RBV dose (Figure 3A). When patients were stratified into two groups according to age, the relapse rate decreased with an increase in RBV dose in patients <60 years. The relapse rate was lowest (11%) in patients <60 years who received ≥ 4.0 g/kg of body weight of RBV. By contrast, among patients ≥ 60 years, the relapse rate decreased with an increase in RBV dose up to 3.0 g/kg of body weight, but remained relatively stable despite a further increase in the RBV dose beyond 3.0 g/kg of body weight. The rate of relapse was 31% to 33% in patients who received ≥ 3.0 g/kg of body weight.

Patients ≥ 60 years had higher relapse rate compared with patients <60 years after stratification by RBV dose ($P=0.044$ for RBV <2.5 g/kg, $P=0.009$ for RBV 2.5–2.9 g/kg, $P=0.150$ for RBV 3.0–3.4 g/kg, $P=0.036$ for RBV 3.5–3.9 g/kg and $P=0.006$ for RBV ≥ 4.0 g/kg).

To exclude the effect of the duration of therapy, patients who received 42–54 weeks of therapy were selected ($n=544$). Again, the relapse rate decreased with an increase in RBV dose in patients <60 years but remained stable despite a further increase in the RBV dose beyond 3.0 g/kg of body weight in patients ≥ 60 years (Figure 3B); in addition, patients ≥ 60 years had a higher relapse rate compared with younger patients after stratification by

Figure 2. Internal and external validation of the decision-tree model: subgroup-stratified comparison of the rate of relapse between the model derivation and validation groups



Each patient in the internal and external validation population was allocated to groups 1 to 5 following the flowchart of the decision tree. The rates of relapse were then calculated for each group and a graph was plotted. The rate of relapse in the (A) internal and (B) external validation groups are shown. The rates of relapse are shown as percentages below data points: the value on the left is from the model derivation group and on the right is from the validation group. The rates of relapse in each group of patients correlated closely between the model derivation group and the validation group (correlation coefficient: $r^2=0.79$ and 0.83 , respectively).

Table 2. Multivariable analysis of factors associated with relapse among patients with RVR/cEVR

Factor	OR	95% CI	P-value
No-RVR	4.07	2.57–6.43	<0.0001
Total RBV dose <3.0 g/kg body weight	2.19	1.58–3.03	<0.0001
Creatinine <0.7 g/dl	1.67	1.22–2.29	0.001
Age ≥ 60 years	2.37	1.73–3.24	<0.0001

cEVR, complete early virological response (HCV-RNA-positive at week 4, but negative at week 12); RBV, ribavirin; RVR, rapid virological response (HCV-RNA-negative at week 4).

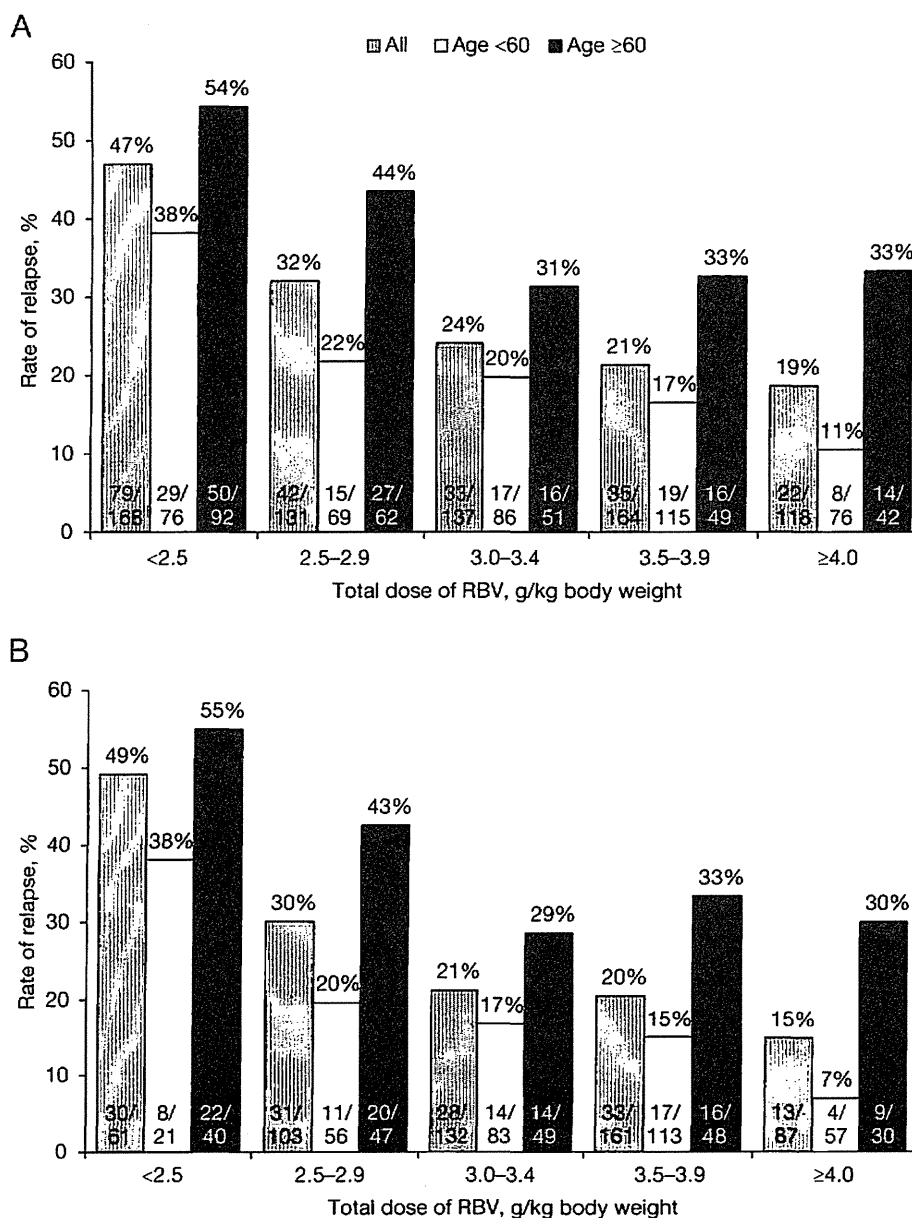
RBV dose ($P=0.283$ for RBV <2.5 g/kg, $P=0.017$ for RBV 2.5–2.9 g/kg, $P=0.127$ for RBV 3.0–3.4 g/kg, $P=0.011$ for RBV 3.5–3.9 g/kg and $P=0.009$ for RBV ≥ 4.0 g/kg).

Total dose of RBV was associated with relapse independently of PEG-IFN dose. The cutoff value of 58 $\mu\text{g}/\text{kg}$ of PEG-IFN was selected, which corresponds to the 80% of 1.5 $\mu\text{g}/\text{kg}$ dose for 48 weeks. In patients who received <58 $\mu\text{g}/\text{kg}$ of body weight of PEG-IFN,

the rate of relapse for patients who received ≥ 3.0 g/kg or <3.0 g/kg of body weight of RBV was 24% and 42%, respectively. In patients who received ≥ 58 $\mu\text{g}/\text{kg}$ of body weight of PEG-IFN, the rate of relapse for patients who received ≥ 3.0 g/kg or <3.0 g/kg of body weight of RBV was 21% and 38%, respectively.

The data mining analysis procedure did not select further split variables among RVR patients. However,

Figure 3. Correlation between the rate of relapse and total RBV dose among patients with cEVR after stratification by age



Association between the total ribavirin (RBV) dose and the rate of relapse among patients with complete early virological response (cEVR) is shown. (A) Higher dose of RBV was associated with reduced rate of relapse. (B) These associations were also confirmed in selected patients who received 42–54 weeks of therapy.

when analysed separately, the rate of relapse was also associated with age and total RBV dose among patients with RVR. The rate of relapse for patients who received ≥ 3.0 g/kg or < 3.0 g/kg of body weight of RBV was 5% and 14%, respectively. The rate of relapse for patients < 60 and ≥ 60 years was 9% and 18%, respectively. Collectively, the rate of relapse for patients < 60 years who received ≥ 3.0 g/kg or < 3.0 g/kg of body weight of RBV was 2% and 11%, respectively, whereas the rate of relapse for patients ≥ 60 years who received ≥ 3.0 g/kg or < 3.0 g/kg of body weight of RBV was 12% and 20%, respectively.

Discussion

The result of the present study shows that older age and insufficient dose of RBV are significant and independent risk factors for relapse among patients with cEVR to PEG-IFN plus RBV. Older patients (≥ 60 years) who received a total RBV dose < 3.0 g/kg of body weight had the highest risk of relapse (52%), whereas younger patients who received a total RBV dose ≥ 3.0 g/kg of body weight had the lowest risk of relapse (16%). The rate of relapse decreased depending on the total RBV dose in younger patients, but remained stable in older patients despite a further increase in the RBV dose beyond 3.0 g/kg of body weight. These findings imply that the target dose of total RBV can be set at 3.0 g/kg of body weight in patients who achieved cEVR, and further increase in RBV dose up to 4.0 g/kg of body weight or greater may be recommended in patients < 60 years.

The associations between the drug adherence and virological response had been reported with inconsistent results. In an earlier study, patients who received $> 80\%$ of the planned dose of PEG-IFN plus RBV for $> 80\%$ of the planned duration of therapy had a higher rate of SVR compared to those who received a lesser dose (51% versus 34%) [31]. Consistent results were obtained in a study reporting that patients who received $> 80\%$ of the planned dose of PEG-IFN and RBV within the first 12 weeks of therapy had a higher rate of EVR compared with those who received a lesser dose of both drugs (80% versus 33%) [4]. By contrast, a large-scale multicentre study showed that reducing the PEG-IFN dose during the first 20 weeks reduced SVR; however, reducing RBV did not affect SVR as long as RBV was not prematurely discontinued [32]. The reason for these inconsistencies is unclear. One reason may be the differences in the backgrounds of patients enrolled in the study, and hence the last study was limited to patients with advanced fibrosis and prior non-responders to PEG-IFN therapy. Because the probability of SVR is affected by virological response and relapse after response, the effect of drug dosing should be analysed separately with respect to these two factors.

In the present study, we focused on factors predictive of relapse after early virological response. According to the decision tree model, relapse was less likely in patients with RVR compared with cEVR. Among patients with cEVR, older patients (≥ 60 years) had a higher risk of relapse compared to younger patients (41% versus 22%). In addition, our results emphasized the effect of RBV dose for the prevention of relapse. In our study, a total RBV dose of ≥ 3.0 g/kg of body weight was repeatedly associated with a suppressed rate of relapse in the model derivation and validation groups. The rate of relapse in patients < 60 years who received an RBV dose of < 3.0 versus ≥ 3.0 g/kg of body weight in the model derivation, internal validation and external validation groups were 32% versus 16%, 27% versus 16%, and 41% versus 16%, respectively. The rate of relapse in patients ≥ 60 years who received an RBV dose of < 3.0 versus ≥ 3.0 g/kg of body weight in the model derivation, internal validation and external validation groups were 52% versus 26%, 45% versus 38%, and 44% versus 22%, respectively. It has been reported that the rate of relapse is suppressed in 48 weeks of IFN plus RBV combination therapy compared to IFN monotherapy, indicating that RBV contributes to the increase in SVR by reducing relapse [2,3]. Another study, focused on the associations between the drug dose reduction and relapse in patients with virological response, found that maintaining RBV dose ≥ 12 mg/kg/day during 48 weeks of treatment, which can be translated into a total dose of 4.0 g/kg of body weight, suppressed relapse [33]. Results of the present study are in accordance with this report.

The importance of drug dosing on reduction in relapse is also supported by the findings that extending therapy from 48 to 72 weeks in patients with delayed virological response improved SVR rates by reducing relapse [9–13]. Apart from these clinical studies, in the real world of clinical practice, duration of therapy is extended – even in patients with cEVR – at the physician's discretion. The relationship between duration of therapy or RBV dose, and relapse among patients with cEVR and treated with various lengths of therapy has not been examined. In the combined group of our study, extending the duration of therapy was not associated with a reduction in relapse rate. Rather, the rate of relapse decreased depending on the total RBV dose. These findings suggest that acquiring a sufficient total RBV dose, either within 48 weeks or by extending the duration of therapy, is essential to prevent relapse among patients with cEVR. The limitation of the present study was that the mean duration of therapy was only 56.3 weeks in patients whose duration of therapy was extended beyond 48 weeks. It is probable that extended duration of therapy was not long enough for the prevention of relapse. Further studies with

longer durations of therapy are necessary to confirm the effect of extended duration of therapy on reduction of relapse among patients with cEVR.

Previous reports did not consider the effects of age in setting the optimal dose of RBV. In the present study, the relapse rate decreased with an increase in RBV dose from <2.5 to 3.0–3.5 g/kg of body weight, but remained relatively stable despite a further increase in the RBV dose in older patients. Thus, a total RBV dose ≥ 3.0 g/kg of body weight should be the target dose for patients ≥ 60 years with cEVR. By contrast, ≥ 3.0 g/kg of body weight of RBV was associated with lower risk of relapse in patients <60 with cEVR (16% versus 32%), and a further increase in RBV dose led to a more profound reduction in relapse rates, as low as 11% in patients who received ≥ 4.0 g/kg of body weight. Thus, a total dose of ≥ 4.0 g/kg of body weight or even greater should be the target dose in patients <60 years.

In the near future, more potent therapies, such as direct antiviral agents [34,35], may become available. These drugs require RBV and PEG-IFN in combination. However, not all patients may be able to tolerate this triple combination therapy due to adverse drug reactions, such as severe anaemia or skin eruption. In particular, it may be difficult to administer a full dose of triple drugs to older patients. Thus, personalizing the PEG-IFN and RBV combination therapy based on this model may be beneficial to patients who were intolerant to triple combination therapy.

In the present study creatinine was an independent predictor of relapse by multivariable logistic regression analysis. However creatinine was not selected as a splitting variable in decision tree, which may be due to the unique property of data mining analysis. In data mining analysis, limitation is imposed to stop the analysis when the number of patients is <20. This limitation is used to avoid dividing patients into too small subgroups which lead to the generation of rules that only apply to the model derivation population and not reproduced when applied to other populations. This phenomenon is called the over-fitting of the model. Due to this limitation, the variables selected in the data mining analysis are not necessarily identical to the variables that are significant by ordinary multivariable analysis. In a separate analysis, lower level of creatinine was associated with higher rate of relapse in each subgroup of patients with cEVR. The reason for this association is not clear, but lower creatinine level may be related to more efficient clearance of RBV leading to lower serum level of RBV. Further research is needed to confirm this speculation.

A potential limitation of the present study is that data mining analysis has an intrinsic risk of showing relationships that fit to the original dataset, but

are not reproducible in different groups. Although internal and external validations showed that our model had high reproducibility, we recognized that further validation on a larger external validation cohort, especially in groups other than Japanese, may be necessary to further verify the reliability of our model.

In conclusion, we built a decision tree model for the prediction of relapse among patients with EVR to PEG-IFN plus RBV. The result of the present study shows that older age and insufficient dose of RBV are significant and independent risk factors for relapse. The target dose of total RBV can be set at 3.0 g/kg of body weight in patients who achieved cEVR. A further increase in RBV dose up to 4.0 g/kg of body weight may be warranted in patients <60 years.

Acknowledgements

This study was supported by a Grant-in-Aid from the Ministry of Health, Labor and Welfare, Japan (H20-kanen-006).

Disclosure statement

The authors declare no competing interests.

References

1. Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; 39:1147–1171.
2. Fried MW, Shiffman ML, Reddy KR, *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347:975–982.
3. Manns MP, McHutchison JG, Gordon SC, *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; 358:958–965.
4. Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003; 38:645–652.
5. Lee SS, Ferenci P. Optimizing outcomes in patients with hepatitis C virus genotype 1 or 4. *Antivir Ther* 2008; 13 Suppl 1:9–16.
6. Namiki I, Nishiguchi S, Hino K, *et al.* Management of hepatitis C: report of the consensus meeting at the 45th annual meeting of the Japan Society of Hepatology (2009). *Hepatol Res* 2010; 40:347–368.
7. Jensen DM, Morgan TR, Marcellin P, *et al.* Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40 kd)/ribavirin therapy. *Hepatology* 2006; 43:954–960.
8. Yu MI, Dai CY, Huang JF, *et al.* Rapid virological response and treatment duration for chronic hepatitis C genotype 1 patients: a randomized trial. *Hepatology* 2008; 47:1884–1893.
9. Berg T, von Wagner M, Nasser S, *et al.* Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. *Gastroenterology* 2006; 130:1086–1097.

10. Sánchez-Tapias JM, Diago M, Escartin P, *et al.* Peginterferon-alfa2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. *Gastroenterology* 2006; 131:451–460.
11. Ferenci P, Laferl H, Scherzer TM, *et al.* Peginterferon alfa-2a/ribavirin for 48 or 72 weeks in hepatitis C genotypes 1 and 4 patients with slow virologic response. *Gastroenterology* 2010; 138:503–512.e1.
12. Buti M, Lurie Y, Zakharova NG, *et al.* Randomized trial of peginterferon alfa-2b and ribavirin for 48 or 72 weeks in patients with hepatitis C virus genotype 1 and slow virologic response. *Hepatology* 2010; 52:1201–1207.
13. Pearlman BL, Ehleben C, Saifee S. Treatment extension to 72 weeks of peginterferon and ribavirin in hepatitis c genotype 1-infected slow responders. *Hepatology* 2007; 46:1688–1694.
14. Tanaka Y, Nishida N, Sugiyama M, *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; 41:1105–1109.
15. Suppiah V, Moldovan M, Ahlenstiel G, *et al.* IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; 41:1100–1104.
16. Ge D, Fellay J, Thompson AJ, *et al.* Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; 461:399–401.
17. Kurosaki M, Tanaka Y, Nishida N, *et al.* Pre-treatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in IL28B and viral factors. *J Hepatol* 2011; 54:439–448.
18. Breiman L, Friedman RA, Olshen CJ, Stone CM. *Classification and regression trees*. 1980. Belmont, CA: Wadsworth.
19. Garzotto M, Park Y, Mongoue-Tchokote S, *et al.* Recursive partitioning for risk stratification in men undergoing repeat prostate biopsies. *Cancer* 2005; 104:1911–1917.
20. Miyaki K, Takei I, Watanabe K, Nakashima H, Omac K. Novel statistical classification model of type 2 diabetes mellitus patients for tailor-made prevention using data mining algorithm. *J Epidemiol* 2002; 12:243–248.
21. Averbook BJ, Fu P, Rao JS, Mansour EG. A long-term analysis of 1018 patients with melanoma by classic Cox regression and tree-structured survival analysis at a major referral center: Implications on the future of cancer staging. *Surgery* 2002; 132:589–604.
22. Leiter U, Buettner PG, Eigentler TK, Garbe C. Prognostic factors of thin cutaneous melanoma: an analysis of the central malignant melanoma registry of the German dermatological society. *J Clin Oncol* 2004; 22:3660–3667.
23. Valera VA, Walter BA, Yokoyama N, *et al.* Prognostic groups in colorectal carcinoma patients based on tumor cell proliferation and classification and regression tree (CART) survival analysis. *Ann Surg Oncol* 2007; 14:34–40.
24. Zlobec I, Steele R, Nigam N, Compton CC. A predictive model of rectal tumor response to preoperative radiotherapy using classification and regression tree methods. *Clin Cancer Res* 2005; 11:5440–5443.
25. Baquerizo A, Anselmo D, Shackleton C, *et al.* Phosphorus ans an early predictive factor in patients with acute liver failure. *Transplantation* 2003; 75:2007–2014.
26. Kurosaki M, Matsunaga K, Hirayama I, *et al.* A predictive model of response to peginterferon ribavirin in chronic hepatitis C using classification and regression tree analysis. *Hepatol Res* 2010; 40:251–260.
27. Kurosaki M, Sakamoto N, Iwasaki M, *et al.* Pretreatment prediction of response to peginterferon plus ribavirin therapy in genotype 1 chronic hepatitis C using data mining analysis. *J Gastroenterol* 2011; 46:401–409.
28. Kurosaki M, Sakamoto N, Iwasaki M, *et al.* Sequences in the interferon sensitivity determining region and core region of hepatitis C virus impact pretreatment prediction of response to peg-interferon plus ribavirin: data mining analysis. *J Med Virol* 2011; 83:445–452.
29. LeBlanc M, Crowley J. A review of tree-based prognostic models. *Cancer Treat Res* 1995; 75:113–124.
30. Segal MR, Bloch DA. A comparison of estimated proportional hazards models and regression trees. *Stat Med* 1989; 8:539–550.
31. McHutchison JG, Manns M, Patel K, *et al.* Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; 123:1061–1069.
32. Shiffman ML, Ghany MG, Morgan TR, *et al.* Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* 2007; 132:103–112.
33. Hiramatsu N, Oze T, Yakushijin T, *et al.* Ribavirin dose reduction raises relapse rate dose-dependently in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2b plus ribavirin. *J Viral Hepat* 2009; 16:586–594.
34. Hézode C, Forestier N, Dusheiko G, *et al.* Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009; 360:1839–1850.
35. McHutchison JG, Everson GT, Gordon SC, *et al.* Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; 360:1827–1838.

Accepted 18 April 2011; published online 21 October 2011

Model Incorporating the *ITPA* Genotype Identifies Patients at High Risk of Anemia and Treatment Failure With Pegylated-Interferon Plus Ribavirin Therapy for Chronic Hepatitis C

Masayuki Kurosaki,¹ Yasuhito Tanaka,² Nao Nishida,³ Naoya Sakamoto,⁴ Nobuyuki Enomoto,⁵ Kentaro Matsuura,² Yasuhiro Asahina,⁶ Mina Nakagawa,⁶ Mamoru Watanabe,⁶ Minoru Sakamoto,⁵ Shinya Maekawa,⁵ Katsushi Tokunaga,³ Masashi Mizokami,⁷ and Namiki Izumi^{1*}

¹Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan

²Department of Virology, Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

³Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

⁴Department of Gastroenterology and Hematology, Hokkaido University, Sapporo, Japan

⁵First Department of Internal Medicine, University of Yamanashi, Yamanashi, Japan

⁶Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, Japan

⁷Research Center for Hepatitis and Immunology, International Medical Center of Japan Konodai Hospital, Ichikawa, Japan

This study aimed to develop a model for predicting anemia using the inosine triphosphatase (*ITPA*) genotype and to evaluate its relationship with treatment outcome. Patients with genotype 1b chronic hepatitis C ($n = 446$) treated with peg-interferon alpha and ribavirin (RBV) for 48 weeks were genotyped for the *ITPA* (rs1127354) and *IL28B* (rs8099917) genes. Data mining analysis generated a predictive model for anemia (hemoglobin (Hb) concentration <10 g/dl); the CC genotype of *ITPA*, baseline Hb <14.0 g/dl, and low creatinine clearance (CLcr) were predictors of anemia. The incidence of anemia was highest in patients with Hb <14.0 g/dl and CLcr <90 ml/min (76%), followed by Hb <14.0 g/dl and *ITPA* CC (57%). Patients with Hb ≥ 14.0 g/dl and *ITPA* AA/CA had the lowest incidence of anemia (17%). Patients with two predictors (high-risk) had a higher incidence of anemia than the others (64% vs. 28%, $P < 0.0001$). At baseline, the *IL28B* genotype was a predictor of a sustained virological response [adjusted odds ratio 9.88 (95% confidence interval 5.01–19.48), $P < 0.0001$]. In patients who achieved an early virological response, the *IL28B* genotype was not associated with a sustained virological response, while a high risk of anemia was a significant negative predictor of a sustained virological response [0.47 (0.24–0.91), $P = 0.026$]. For high-risk patients with an early virological response, giving $>80\%$ of the planned RBV dose increased sustained virological responses by 24%. In conclusion, a predictive model

incorporating the *ITPA* genotype could identify patients with a high risk of anemia and reduced probability of sustained virological response.

J. Med. Virol. 85:449–458, 2013.

© 2013 Wiley Periodicals, Inc.

KEY WORDS: hemolytic anemia; ribavirin; creatinine clearance; antiviral therapy

INTRODUCTION

Hepatitis C virus (HCV) infection is a leading cause of cirrhosis and hepatocellular carcinoma worldwide [Kim, 2002]. The rate of eradication of HCV by pegylated interferon (PEG-IFN) plus ribavirin (RBV), defined as a sustained virological response, is around 50% in patients with HCV genotype 1 [Manns et al., 2001; Fried et al., 2002]. Failure of treatment is attributable to the lack of a virological response or relapse after completion of therapy. Genome-wide association studies and subsequent cohort studies

Grant sponsor: Ministry of Health, Labor and Welfare, Japan.

Conflicts of interest and financial disclosures: None reported.

*Correspondence to: Namiki Izumi, MD, PhD, Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, 1-26-1 Kyonan-cho, Musashino-shi, Tokyo 180-8610, Japan. E-mail: nizumi@musashino.jrc.or.jp

Accepted 19 November 2012

DOI 10.1002/jmv.23497

Published online 7 January 2013 in Wiley Online Library (wileyonlinelibrary.com).

have shown that single nucleotide polymorphisms (SNPs) located near the *IL28B* gene are the most important determinant of virological response to PEG-IFN/RBV therapy [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Rauch et al., 2010]. On the other hand, among patients with a virological response, the probability of a sustained virological response decreases when the patients become intolerant to therapy because of RBV-induced hemolytic anemia and receive a reduced dose of RBV [McHutchison et al., 2002; Kurosaki et al., 2012]. Genome-wide association studies have shown that variants of the inosine triphosphatase (*ITPA*) gene protect against hemolytic anemia [Fellay et al., 2010; Tanaka et al., 2011]. These variants are associated with a reduced requirement for an anemia-related dose reduction of RBV [Sakamoto et al., 2010; Thompson et al., 2010a; Kurosaki et al., 2011d; Seto et al., 2011]. However, factors other than the *ITPA* gene also contribute to the risk of severe anemia or RBV dose reduction [Ochi et al., 2010; Kurosaki et al., 2011d] and the results of studies on the impact of the *ITPA* genotype on treatment outcome are inconsistent [Ochi et al., 2010; Sakamoto et al., 2010; Thompson et al., 2010a, 2011; Kurosaki et al., 2011d].

Data mining is a novel statistical method used to extract relevant factors from a plethora of factors and combine them to predict the incidence of the outcome of interest [Breiman et al., 1980]. Decision tree analysis, a primary component of data mining analysis, has found medical applications recently [Averbook et al., 2002; Miyaki et al., 2002; Baquerizo et al., 2003; Leiter et al., 2004; Garzotto et al., 2005; Zlobec et al., 2005; Valera et al., 2007] and has proven to be a useful tool for predicting therapeutic efficacy [Kurosaki et al., 2010, 2011a,b,c, 2012] and adverse events [Hiramatsu et al., 2011] in patients with chronic hepatitis C treated with PEG-IFN/RBV therapy. Because the results of data mining analysis are presented as a flowchart [LeBlanc and Crowley, 1995], they are easily understandable and usable by clinicians lacking a detailed knowledge of statistics.

For the general application of this genetic information in clinical practice, this study aimed to construct a predictive model of severe anemia using the *ITPA* genotype, together with other relevant factors. This study also aimed to analyze the impact of the risk of anemia on treatment outcome, after adjustment for the *IL28B* genotype. These analyses were carried out at baseline and during therapy, when the early virological response became evident.

MATERIALS AND METHODS

Patients

Data were collected from a total of 446 genotype 1b chronic hepatitis C patients who were treated with PEG-IFN alpha and RBV at five hospitals and universities throughout Japan. The inclusion criteria were: (1) infection by hepatitis C genotype 1b; (2) no

co-infection with hepatitis B virus or human immunodeficiency virus; (3) no other causes of liver disease such as autoimmune hepatitis and primary biliary cirrhosis; and (4) availability of DNA for the analysis of the genetic polymorphisms of *IL28B* and *ITPA*. Patients received PEG-IFN alpha-2a (180 µg) and 2b (1.5 µg/kg) subcutaneously every week and a daily weight-adjusted dose of RBV (600 mg for patients weighing <60 kg, 800 mg for patients weighing 60–80 kg, and 1,000 mg for patients weighing >80 kg) for 48 weeks. Dose reduction or discontinuation of PEG-IFN and RBV was primarily based on the recommendations on the package inserts and the discretion of the physicians at each university and hospital. The standard duration of therapy was set at 48 weeks. No patient received erythropoietin or other growth factors for the treatment of anemia. Written informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committees.

Laboratory Tests

Blood samples obtained before therapy were analyzed for hematologic data, blood chemistry, and HCV RNA. Genetic polymorphisms in SNPs of the *ITPA* gene (rs1127354) and the *IL28B* gene (rs8099917) were determined using ABI TaqMan Probes (Applied Biosystems, Carlsbad, CA) and the DigiTag2 assay, respectively. Baseline creatinine clearance (CLcr) levels were calculated using the formula of Cockcroft and Gault [1976]: for males, $CLcr = [(140 - \text{age in years}) \times \text{body weight in kg}] \div (72 \times \text{serum creatinine in mg/dl})$ and for females, $CLcr = 0.85 \times [(140 - \text{age in years}) \times \text{body weight in kg}] \div (72 \times \text{serum creatinine in mg/dl})$. The stage of liver fibrosis was scored according to the METAVIR scoring system: F0 (no fibrosis), F1 (mild fibrosis: portal fibrosis without septa), F2 (moderate fibrosis: few septa), F3 (severe fibrosis: numerous septa without cirrhosis), and F4 (cirrhosis). A rapid virological response was defined as undetectable HCV RNA by qualitative PCR with a lower detection limit of 50 IU/ml (Amplicor, Roche Diagnostic Systems, Pleasanton, CA) at week 4 of therapy and a complete early virological response was defined as undetectable HCV RNA at week 12. A sustained virological response was defined as undetectable HCV RNA at 24 weeks after completion of therapy. Severe anemia was defined as hemoglobin (Hb) <10 g/dl.

Statistical Analysis

Database for analysis included the following variables: age, sex, body mass index, serum aspartate aminotransferase (AST) levels, alanine aminotransferase (ALT) levels, gamma-glutamyltransferase (GGT) levels, creatinine levels, CLcr, Hb, platelet count, serum levels of HCV RNA, and the stage of liver fibrosis

TABLE I. Patients' Baseline Characteristics

Age (years)	58.6	(9.6)
Gender: male (n, %)	185	(42%)
Body mass index (kg/m ²)	23.1	(3.7)
AST (IU/L)	59.9	(53.8)
ALT (IU/L)	69.8	(53.8)
GGT (IU/L)	48.5	(41.6)
Creatinine (mg/dl)	0.7	(0.2)
Creatinine clearance (ml/min)	89.5	(23.0)
Hemoglobin (g/dl)	14	(1.4)
Platelet count (10 ⁹ /L)	154.5	(52.1)
HCV RNA > 600,000 IU/ml (n, %)	354	(79%)
Liver fibrosis: F3-4 (n, %)	108	(24%)
Initial ribavirin dose (n, %)		
600 mg/day	300	(67%)
800 mg/day	138	(31%)
1,000 mg/day	9	(2%)
Pegylated interferon (n, %)		
alpha2a 180 mcg	58	(13%)
alpha2b 1.5 mcg/kg	388	(87%)
<i>ITPA</i> rs1127354: CC (n, %)	317	(71%)
<i>IL28B</i> rs809917: TT (n, %)	311	(70%)

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase. Data expressed as mean (standard deviation) unless otherwise mentioned.

(Table I). Based on these data set, a model for predicting the risk of developing severe anemia was constructed by data mining analysis using the IBM-SPSS Modeler 13 as described previously [Kurosaki et al., 2010, 2011a,b,c; Hiramatsu et al., 2011]. Briefly, the software was used to explore the database automatically to search for optimal predictors that discriminated most efficiently patients with severe anemia from those without. The software also determined the optimal cutoff values of each predictor. Patients were divided into two groups according to the predictor and each of the two groups was repeatedly divided in the same way until no significant factor remained or 20 or fewer patients were in a group.

The incidence of severe anemia, the total dose of RBV, and treatment outcome were compared between groups with high and low risks of anemia. On univariate analysis, Student's *t*-test was used for continuous variables, and Fisher's exact test was used for categorical data. Logistic regression was used for multivariate analysis. *P* values of <0.05 were considered significant. SPSS Statistics 18 was used for these analyses.

RESULTS

Predictive Model of Severe Anemia

The incidence of severe anemia in the whole cohort was 49% (Fig. 1). The best predictor of severe anemia was the baseline Hb concentration. Patients with a low baseline Hb concentration (<14 g/dl) were more likely to develop severe anemia (67%) than those with a higher Hb (>14 g/dl) (34%). The second best predictor for those patients with a baseline Hb <14.0 g/dl was CLcr. Patients with a CLcr below 90 ml/min had

the highest incidence of severe anemia (76%). In those with a CLcr above >90 ml/min the incidence of severe anemia was 57% in patients with the CC allele of the *ITPA* gene while it was 37% in patients with the CA or AA allele. On the other hand, the second best predictor for those patients with a baseline Hb concentration above 14 g/dl was the *ITPA* genotype. Patients with the AA or AC allele had the lowest incidence of anemia (17%). For those with the *ITPA* CC allele, CLcr was the third best predictor; the optimal cutoff value was 85 ml/min for this group. The incidence of severe anemia was 49% in patients with a CLcr below 85 ml/min while it was 32% in those with a CLcr above 85 ml/min.

Following this analysis, the patients were divided into six groups, with the incidence of severe anemia ranging from 17% to 76%. Three groups with two predictors, having an incidence of anemia >40%, were defined as the high-risk group and the remainder were defined as the low-risk group. The incidence of severe anemia was higher in the high-risk group than the low-risk group (65% vs. 28%, *P* = 0.029) (Fig. 2). Comparison of the *ITPA* genotype and the predictive model showed that the sensitivity for the prediction of severe anemia was similar (75.9% vs. 76.4%) but the specificity of the predictive model was greater (33.6% vs. 59.3%).

The Risk of Anemia Impacts on Sustained Virological Responses by Patients Who Achieved an Early Virological Response

The impact of *IL28B* genotype, *ITPA* genotype, and risk group of anemia on the rate of sustained virological response was studied at baseline and week 12. At baseline, patients with the TT allele of the *IL28B* gene had a significantly higher rate of sustained virological response than those with the TG or GG allele (43% vs. 10%, *P* < 0.0001), the high-risk group for anemia had a significantly lower rate of sustained virological response than the low-risk group (28% vs. 40%, *P* = 0.011), and the *ITPA* genotype was not associated with a sustained virological response (Fig. 3A-C). At week 4, patients with rapid virological response had a high rate of sustained virological response, irrespective of the *IL28B* genotype (TT vs. TG/GG; 97% vs. 100%, *P* = 1.000), the *ITPA* genotype (CC vs. CA/AA; 95% vs. 100%, *P* = 1.000), and the risk of anemia (high vs. low; 95% vs. 100%, *P* = 1.000). Among the patients who did not achieve a rapid virological response, those with the *IL28B* TT allele had a significantly higher rate of sustained virological response than those with the TG or GG allele (38% vs. 8%, *P* < 0.0001), and the high-risk group for anemia had a significantly lower rate of sustained virological response than the low-risk group (24% vs. 35%, *P* = 0.015). At week 12, in patients who achieved a complete early virological response, the *IL28B* genotype was not associated with a sustained virological response, while the high-risk group for anemia had a

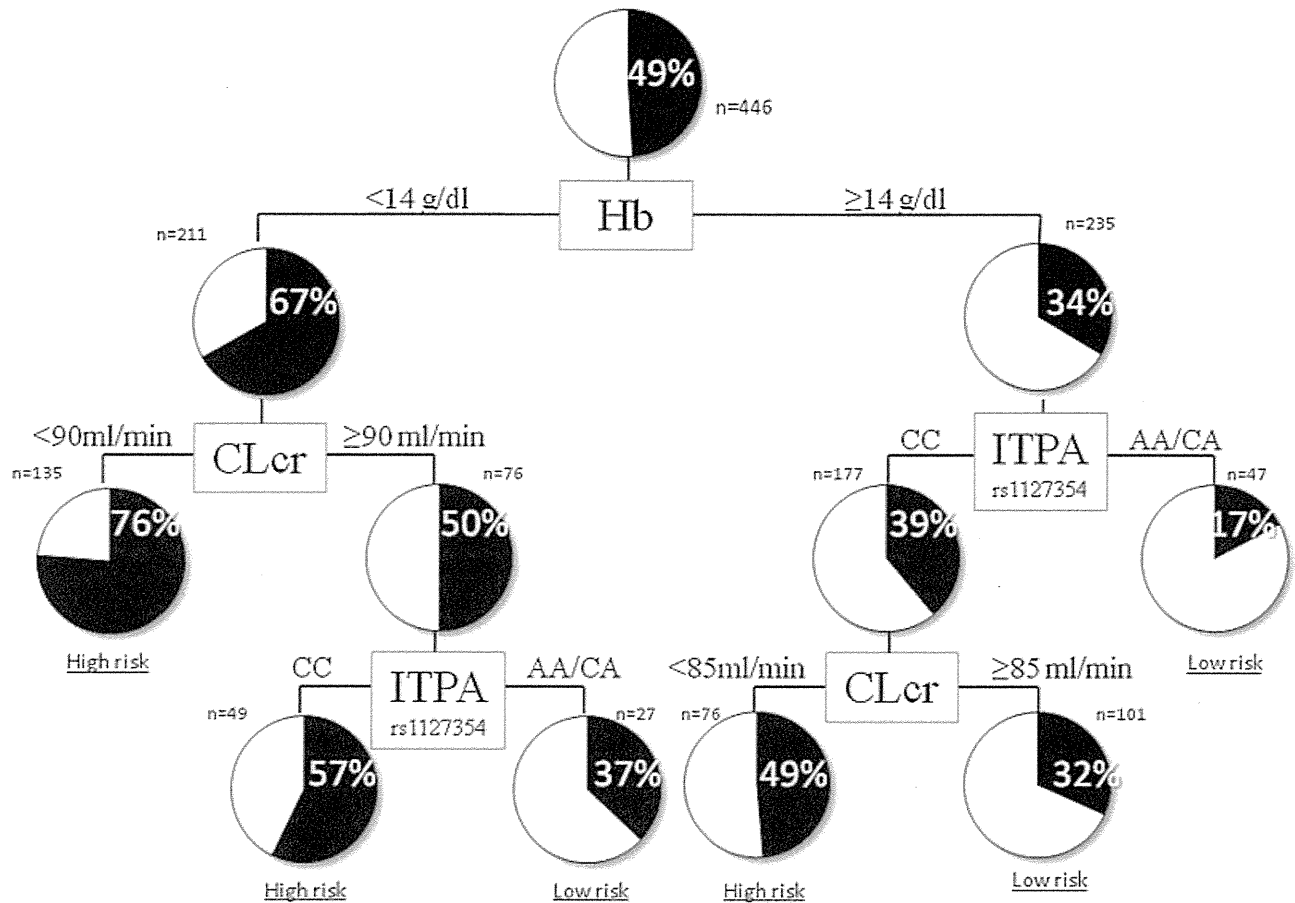


Fig. 1. The predictive model for severe anemia. The boxes indicate the factors used to differentiate patients and the cutoff values for the different groups. The pie charts indicate the rate of severe anemia (Hb <10.0 g/dl) for each group of patients, after differentiation. Terminal groups of patients differentiated by analysis are classified as at high risk if the rate is >40% and low risk if the rate is <40%. ITPA, inosine triphosphatase; CLcr, creatinine clearance; Hb, hemoglobin.

significantly lower rate of sustained virological response than the low-risk group (59% vs. 76%, $P = 0.013$) (Fig. 3D–F). In patients who did not achieve a complete early virological response, the *IL28B* genotype was a significant predictor of a sustained virological response (TT vs. TG/GG; 14% vs. 2%, $P < 0.0001$) but a high risk for anemia was not (high vs. low; 10% vs. 6%, $P = 0.361$).

From multivariate analysis (Table II), the *IL28B* genotype was the most important predictor of a sustained virological response at baseline [adjusted odds ratio 9.88 (95% confidence interval 5.01–19.48), $P < 0.0001$], along with female sex [0.42 (0.26–0.68), $P < 0.0001$], platelet count [1.09 (1.04–1.15), $P < 0.0001$], advanced fibrosis [0.49 (0.27–0.91), $P = 0.024$], and baseline HCV RNA load [4.14 (2.27–7.55), $P < 0.0001$]. At week 4, in patients without a rapid virological response, the *IL28B* genotype remained the most important predictor of a sustained virological response [7.16 (3.60–14.25), $P < 0.0001$], along with female sex and platelet count. At week 12, in patients with a complete early virological response, the risk of anemia was an independent and significant

predictor of a sustained virological response [0.47 (0.24–0.91), $P = 0.026$], together with the platelet count and HCV RNA load, but the *IL28B* genotype was not associated with a sustained virological response. In patients without a complete early virological response, the *IL28B* genotype was a predictor of a sustained virological response [9.13 (2.02–41.3), $P = 0.004$] along with the platelet count. Thus, *IL28B* was a significant predictor of a sustained virological response at baseline and among virological non-responders at weeks 4 and 12. On the other hand, once a complete early virological response was achieved, the *IL28B* genotype was no longer associated with a sustained virological response but the risk of anemia was an independent predictor of a sustained virological response.

The Risk of Anemia, RBV Dose, and Treatment Outcome in Patients With a Complete Early Virological Response

Patients who achieved a complete early virological response were stratified according to adherence to

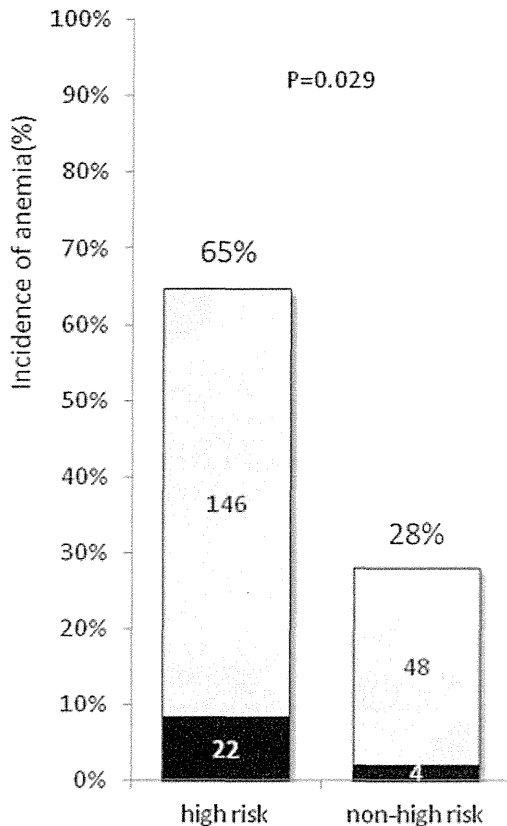


Fig. 2. The incidence of severe anemia stratified by risk of anemia. The incidence of anemia during therapy is shown for each group of patients at high and low risk of anemia. The black and white bars represent the percentages of patients with Hb concentrations below 8.5 g/dl and above 10 g/dl, respectively.

RBV ($\leq 40\%$, 41–60%, 61–80%, and $>80\%$), which showed that patients with a high risk of anemia were predominantly in subgroups with a lower adherence to RBV ($\leq 40\%$, 41–60%, and 61–80%), whereas patients with a low risk of anemia were predominantly in subgroups with a higher adherence to RBV ($>80\%$) (Fig. 4, upper panel). The percentage of patients who received $>80\%$ of the planned dose of RBV was significantly higher in the low-risk group for anemia than in the high-risk group (74% vs. 55%, $P < 0.0001$).

Within the groups with high and low risks of anemia, there was a stepwise increase in the rate of sustained virological response according to the increase in adherence to RBV (Fig. 4, lower panel). The rate of sustained virological response was higher in patients who received $>80\%$ of the planned dose of RBV than those who received less, for both high-risk patients (71% vs. 47%, $P = 0.016$) and low-risk patients (81% vs. 60%, $P = 0.072$). Within the same subgroup of RBV adherence, however, the rate of sustained virological response did not differ between patients with a high risk and a low risk of anemia. Taken together, these results suggest that patients with a high risk of anemia have a disadvantage because they are likely

to be intolerant to RBV, leading to reduced adherence to RBV throughout the 48 weeks of therapy and a reduced rate of sustained virological response. However, if $>80\%$ adherence to RBV could be obtained, the rate of sustained virological response would increase by 24%.

DISCUSSION

This study confirmed previous reports that the *IL28B* genotype is the most significant predictor of a sustained virological response to PEG-IFN plus RBV therapy in chronic hepatitis C patients at baseline [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Rauch et al., 2010; Kurosaki et al., 2011c] and at week 4 [Thompson et al., 2010b], but it had no impact on the rate of sustained virological response among those patients who achieved a complete early virological response [Thompson et al., 2010b; Kurosaki et al., 2011c]. In contrast, the risk of anemia, assessed by the combination of the *ITPA* genotype, baseline Hb concentration, and baseline CLcr, was found to be associated with a sustained virological response in patients who achieved a complete early virological response. Generally, a complete early virological response is the hallmark of a high probability of a sustained virological response, but the rate of sustained virological responses in patients who achieved a complete early virological response and had a high risk of anemia was as low as 59%. This reduced rate of sustained virological response in these patients was attributable to poor adherence to RBV throughout the 48 weeks of therapy. Because administration of $>80\%$ of the planned RBV dose increased the rate of sustained virological response by 24%, it may be postulated that personalizing the treatment schedule to achieve a sufficient dose of RBV, such as extension of treatment duration, may improve sustained virological response rates in these patients. Clearly, this postulate needs to be confirmed in future study. Thus, the findings presented here may have the potential to support selection of the optimum, personalized treatment strategy for an individual patient, based on the risk of anemia.

The degree of hemolytic anemia caused by RBV varies among individuals. A reduction of the Hb concentration early during therapy predicts the likely development of severe anemia [Hiramatsu et al., 2008, 2011] but there are no reliable predictors at baseline. A breakthrough came from the results of a genome-wide association study that revealed that variants of the *ITPA* gene are protective against hemolytic anemia [Fellay et al., 2010]. The *ITPA* genotype has been shown repeatedly to be associated with the degree of hemolytic anemia and dose reduction of RBV [Fellay et al., 2010; Sakamoto et al., 2010; Thompson et al., 2010a; Seto et al., 2011; Tanaka et al., 2011; Kurosaki et al., 2011d]. However, factors other than the *ITPA* gene, such as baseline Hb concentrations [Ochi et al., 2010; Kurosaki et al., 2011d], platelet counts [Ochi

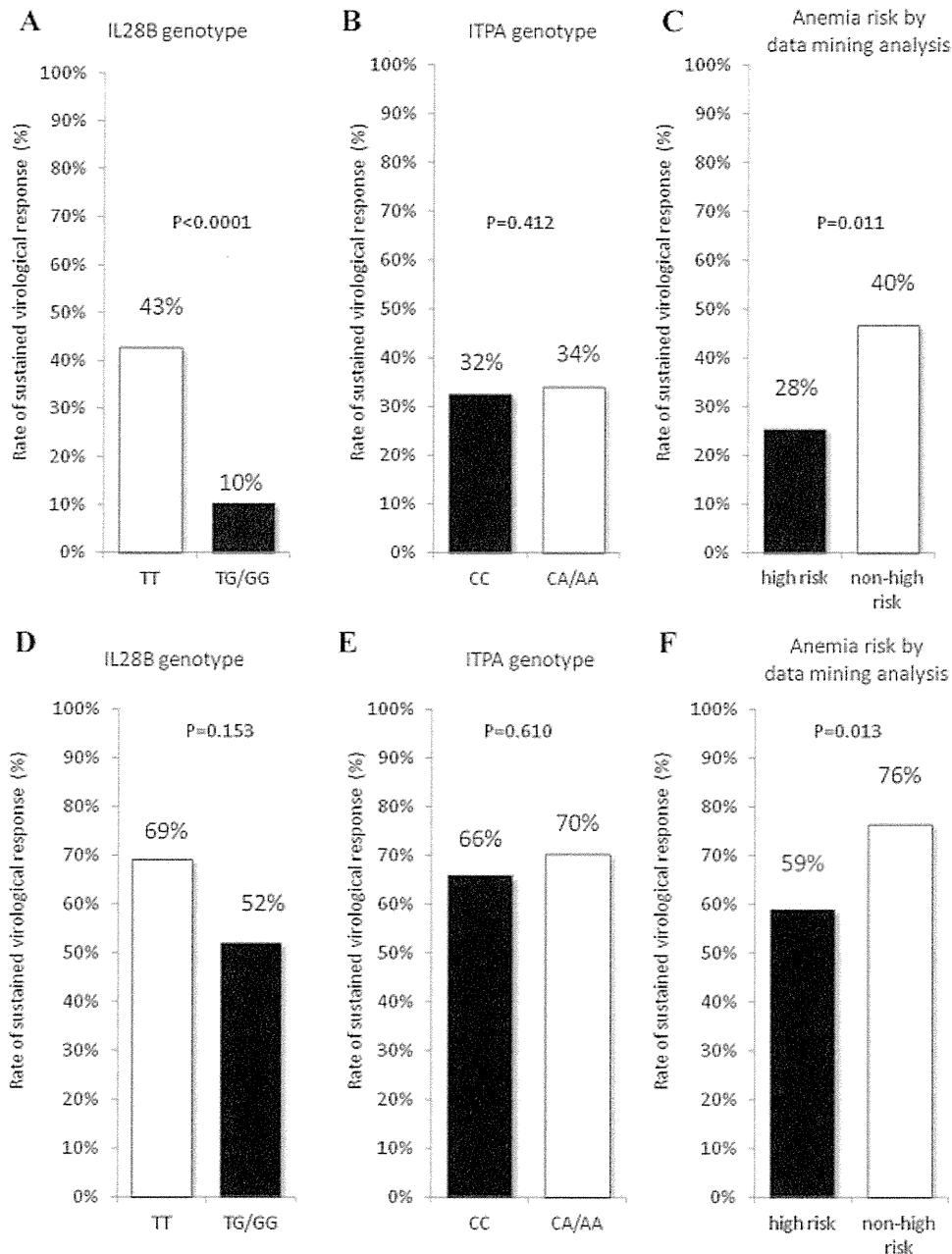


Fig. 3. Rates of sustained virological responses at baseline and among those with a virological response at week 12. The impacts of *IL28B* genotype, *ITPA* genotype, and risk group of anemia on the rate of sustained virological response were studied at baseline (A–C) and among those with complete early virological responses (defined as undetectable HCV RNA at week 12) (D–F). At baseline, those with the TT allele of the *IL28B* gene had a significantly higher rate of sustained virological response than those with the TG or GG allele and the group at high-risk of anemia had a significantly lower rate of sustained virological response than the low-risk group. Among patients with complete early virological responses, the *IL28B* genotype was not associated with a sustained virological response, while the group at high-risk of anemia had a significantly lower rate of sustained virological response than the low-risk group.

et al., 2010], and CLcr [Kurosaki et al., 2011d], also contribute to the risk of severe anemia or RBV dose reduction. In the present study, the predictive model of anemia based on the data mining analysis selected the *ITPA* genotype, baseline Hb concentration, and

baseline CLcr as predictive factors and identified six subgroups of patients with a variable rate of severe anemia, ranging from 17% to 76%. The specificity of the prediction of severe anemia was improved by 25.7% in the predictive model, compared to *ITPA*

TABLE II. Logistic Regression Analysis for Factors Associated With Sustained Virological Response at Baseline, Week 4 and Week 12

	Multi-variable		
	Odds	95% CI	P-value
Pre-treatment			
Sex: female	0.42	0.26–0.68	<0.0001
Platelet (10 ⁹ /L)	1.09	1.04–1.15	<0.0001
Fibrosis: F3-4	0.49	0.27–0.91	0.024
HCV RNA: <600,000 IU/L	4.14	2.27–7.55	<0.0001
<i>IL28B</i> rs8099917: TT	9.88	5.01–19.48	<0.0001
At week 4			
Non-RVR patients			
Sex: female	0.45	0.28–0.72	0.001
Platelet (10 ⁹ /L)	1.10	1.05–1.16	0.000
<i>IL28B</i> rs8099917: TT	7.16	3.60–14.25	<0.0001
At week 12			
cEVR patients			
Platelet (10 ⁹ /L)	1.09	1.02–1.17	0.015
HCV RNA: <600,000 IU/L	3.21	1.39–7.55	0.007
High-risk of anemia ^a	0.47	0.24–0.91	0.026
At week 12			
Non-cEVR patients			
Platelet (10 ⁹ /L)	1.11	1.02–1.21	0.017
<i>IL28B</i> rs8099917: TT	9.13	2.02–41.3	0.004

RVR: rapid virological response, defined as undetectable HCV RNA at week 4.

cEVR: complete early virological response, defined as undetectable HCV RNA at week 12.

^aHigh-risk of anemia defined by decision tree analysis includes the following groups: (1) baseline hemoglobin <14.0 g/dl and creatinine clearance <90 ml/min, (2) baseline hemoglobin <14.0 g/dl, creatinine clearance ≥90 ml/min and *ITPA* rs1127354 genotype CC, and (3) baseline hemoglobin ≥14.0 g/dl, *ITPA* rs1127354 genotype CC, and creatinine clearance <85 ml/min.

genotyping alone. Because hemolytic anemia induced by RBV is one of the major adverse events leading to premature termination of therapy [Fried et al., 2002], a method to predict the risk of severe anemia before treatment is important clinically. A predictive model of anemia may have the potential to support individualized treatment strategies; patients at high risk of anemia may be tested intensively for anemia or may be candidates for erythropoietin therapy, whereas those with a low risk of anemia may be treated with a higher dose of RBV. Prediction of anemia will remain important in the era of direct antiviral agents for chronic hepatitis C, because these newer therapies still require RBV and PEG-IFN in combination, and the degree of anemia complicating these therapies may be even greater than with the current combination therapy [McHutchison et al., 2009; Kwo et al., 2010].

Studies of the impact of the *ITPA* genotype on treatment outcome have produced conflicting results. Previous studies of American [Thompson et al., 2010a] and Italian [Thompson et al., 2011] cohorts did not find any association between the *ITPA* genotype and treatment outcome, whereas a marginal difference was observed in a report from Japan [Ochi et al., 2010]. Moreover, with a subgroup analysis of Japanese patients, the variant of the *ITPA* gene was

associated with a sustained virological response in patients with the *IL28B* major genotype [Kurosaki et al., 2011d], in patients infected with HCV other than genotype 1 [Sakamoto et al., 2010], and in patients with pre-treatment Hb concentrations between 13.5 and 15 g/dl [Azakami et al., 2011]. These inconsistent results may be because the impact of anemia may be greater on a cohort of aged patients, such as in Japan. Another reason may be that the *ITPA* genotype is not the sole determinant of anemia; the *ITPA* genotype alone was not associated with treatment outcome in the present study but a high-risk of anemia, defined by the combination of the *ITPA* genotype, baseline Hb concentration, and baseline CLcr, was associated with sustained virological responses by patients with complete early virological responses, even after adjustment for the *IL28B* genotype and other relevant factors. This is in contrast to the finding that the *IL28B* genotype is an independent and significant predictor at baseline of a sustained virological response by patients without a rapid virological response and those without a complete early virological response, but not those with a complete early virological response. These results indicate that the *IL28B* genotype could be used to predict a sustained virological response at baseline or during therapy in patients in whom HCV RNA has not yet become undetectable, but it has no predictive value in patients in whom HCV RNA has become undetectable. The risk of anemia may be used to predict sustained virological responses in a selected subgroup of patients who achieve a complete early virological response.

Patients who received more than 80% of the planned dose of PEG-IFN or RBV had a higher rate of sustained virological responses than those who received a lower cumulative dose [McHutchison et al., 2002; Davis et al., 2003]. Patients who achieve a complete early virological response usually have a good chance of a sustained virological response and the treatment duration is not extended beyond 48 weeks. However, reduced adherence to drugs in these patients was related to relapse after the completion of 48 weeks of therapy [Hiramatsu et al., 2009; Kurosaki et al., 2012]. In the present study, the rate of sustained virological response was 59% in patients who achieved a complete early virological response but had a high risk of anemia, 17% lower than in patients with a low risk of anemia. However, there was a step-wise increase in the rate of sustained virological response according to the increase in adherence to RBV, and the rate of sustained virological response was higher in high-risk patients who received >80% of the planned dose of RBV (71% vs. 47%). This 24% increase in sustained virological response was observed among the patients in the present study who received 48 weeks of treatment. These findings suggest that receiving a sufficient RBV dose is essential for patients with a complete early virological response to attain a sustained virological response and that the treatment strategy should be personalized for patients with a

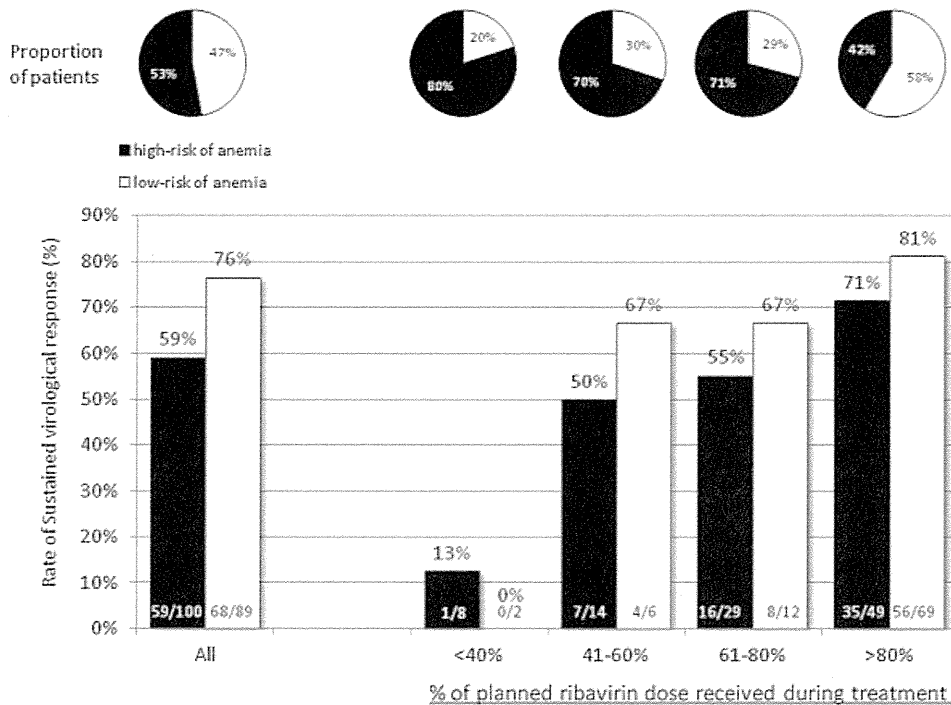


Fig. 4. The impact of risk of anemia and RBV dose on treatment outcome after a complete early virological response. Patients with complete early virological responses were divided into subgroups according to their adherence to RBV: <40%, 41–60%, 61–80%, and >80%. For each subgroup, the proportion of patients with a high risk and a low risk of anemia is shown in the upper panel by pie charts, and the rates of sustained virological responses, stratified by high risk and low risk of anemia, are shown in the lower panel by bar graphs. The black and white bars or charts represent patients with high and low risks of anemia, respectively.

high risk of anemia to extend the duration of treatment, even those patients with a complete early virological response, to obtain >80% adherence to RBV.

In conclusion, the combination of the *ITPA* genotype, baseline Hb concentration, and baseline CLcr could be used as a pre-treatment predictor of anemia. The risk of anemia thus identified is associated with adherence to RBV and impacts on the treatment outcome of patients who achieve a complete early virological response. This is in contrast to the major role of the *IL28B* genotype in the prediction of sustained virological responses at baseline and among non-responders at weeks 4 and 12. Patients who achieve a complete early virological response generally have a high probability of a sustained virological response but those who have a high risk of anemia have a high rate of relapse because of reduced adherence to RBV. To improve the rate of sustained virological responses in these patients, it may be postulated that the treatment schedule may be personalized to obtain >80% adherence to RBV. Clearly, this postulate needs to be confirmed in a future study.

REFERENCES

Averbook BJ, Fu P, Rao JS, Mansour EG. 2002. A long-term analysis of 1018 patients with melanoma by classic Cox regression and tree-structured survival analysis at a major referral

center: Implications on the future of cancer staging. *Surgery* 132:589–602.

Azakami T, Hayes CN, Sezaki H, Kobayashi M, Akuta N, Suzuki F, Kumada H, Abe H, Miki D, Tsuge M, Imamura M, Kawakami Y, Takahashi S, Ochi H, Nakamura Y, Kamatani N, Chayama K. 2011. Common genetic polymorphism of *ITPA* gene affects ribavirin-induced anemia and effect of peg-interferon plus ribavirin therapy. *J Med Virol* 83:1048–1057.

Baquerizo A, Anselmo D, Shackleton C, Chen TW, Cao C, Weaver M, Gornbein J, Geevarghese S, Nissen N, Farmer D, Demetriou A, Busuttill RW. 2003. Phosphorus and an early predictive factor in patients with acute liver failure. *Transplantation* 75:2007–2014.

Breiman LJH, Friedman RA, Olshen CJ, Stone CM. 1980. Classification and regression trees. Calif: Wadsworth.

Cockcroft DW, Gault MH. 1976. Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31–41.

Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. 2003. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 38:645–652.

Fellay J, Thompson AJ, Ge D, Gumbs CE, Urban TJ, Shianna KV, Little LD, Qiu P, Bertelsen AH, Watson M, Warner A, Muir AJ, Brass C, Albrecht J, Sulikowski M, McHutchison JG, Goldstein DB. 2010. *ITPA* gene variants protect against anaemia in patients treated for chronic hepatitis C. *Nature* 464: 405–408.

Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncalves FL, Jr., Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. 2002. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 347:975–982.

Garzotto M, Park Y, Mongoue-Tchokote S, Bledsoe J, Peters L, Blank BH, Austin D, Beer TM, Mori M. 2005. Recursive partitioning for

- risk stratification in men undergoing repeat prostate biopsies. *Cancer* 104:1911–1917.
- Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. 2009. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 461:399–401.
- Hiramatsu N, Kurashige N, Oze T, Takehara T, Tamura S, Kasahara A, Oshita M, Katayama K, Yoshihara H, Imai Y, Kato M, Kawata S, Tsubouchi H, Kumada H, Okanou T, Kakumu S, Hayashi N. 2008. Early decline of hemoglobin can predict progression of hemolytic anemia during pegylated interferon and ribavirin combination therapy in patients with chronic hepatitis C. *Hepatology* 38:52–59.
- Hiramatsu N, Oze T, Yakushiji T, Inoue Y, Igura T, Mochizuki K, Imanaka K, Kaneko A, Oshita M, Hagiwara H, Mita E, Nagase T, Ito T, Inui Y, Hijioka T, Katayama K, Tamura S, Yoshihara H, Imai Y, Kato M, Yoshida Y, Tatsumi T, Ohkawa K, Kiso S, Kanto T, Kasahara A, Takehara T, Hayashi N. 2009. Ribavirin dose reduction raises relapse rate dose-dependently in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2b plus ribavirin. *J Viral Hepat* 16:586–594.
- Hiramatsu N, Kurosaki M, Sakamoto N, Iwasaki M, Sakamoto M, Suzuki Y, Sugauchi F, Tamori A, Kakinuma S, Matsuura K, Izumi N. 2011. Pretreatment prediction of anemia progression by pegylated interferon alpha-2b plus ribavirin combination therapy in chronic hepatitis C infection: Decision-tree analysis. *J Gastroenterol* 46:1111–1119.
- Kim RW. 2002. Global epidemiology and burden of hepatitis C. *Microbes Infect* 4:1219–1225.
- Kurosaki M, Matsunaga K, Hirayama I, Tanaka T, Sato M, Yasui Y, Tamaki N, Hosokawa T, Ueda K, Tsuchiya K, Nakanishi H, Ikeda H, Itakura J, Takahashi Y, Asahina Y, Higaki M, Enomoto N, Izumi N. 2010. A predictive model of response to peginterferon ribavirin in chronic hepatitis C using classification and regression tree analysis. *Hepatology* 40:251–260.
- Kurosaki M, Sakamoto N, Iwasaki M, Sakamoto M, Suzuki Y, Hiramatsu N, Sugauchi F, Tamori A, Nakagawa M, Izumi N. 2011a. Sequences in the interferon sensitivity-determining region and core region of hepatitis C virus impact pretreatment prediction of response to PEG-interferon plus ribavirin: Data mining analysis. *J Med Virol* 83:445–452.
- Kurosaki M, Sakamoto N, Iwasaki M, Sakamoto M, Suzuki Y, Hiramatsu N, Sugauchi F, Yatsushashi H, Izumi N. 2011b. Pretreatment prediction of response to peginterferon plus ribavirin therapy in genotype 1 chronic hepatitis C using data mining analysis. *J Gastroenterol* 46:401–409.
- Kurosaki M, Tanaka Y, Nishida N, Sakamoto N, Enomoto N, Honda M, Sugiyama M, Matsuura K, Sugauchi F, Asahina Y, Nakagawa M, Watanabe M, Sakamoto M, Maekawa S, Sakai A, Kaneko S, Ito K, Masaki N, Tokunaga K, Izumi N, Mizokami M. 2011c. Pretreatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in IL28B and viral factors. *J Hepatol* 54:439–448.
- Kurosaki M, Tanaka Y, Tanaka K, Suzuki Y, Hoshioka Y, Tamaki N, Kato T, Yasui Y, Hosokawa T, Ueda K, Tsuchiya K, Kuzuya T, Nakanishi H, Itakura J, Takahashi Y, Asahina Y, Matsuura K, Sugauchi F, Enomoto N, Nishida N, Tokunaga K, Mizokami M, Izumi N. 2011d. Relationship between genetic polymorphisms in the inosine triphosphatase gene and anemia or outcome after treatment with pegylated-interferon and ribavirin. *Antivir Ther* 16:685–694.
- Kurosaki M, Hiramatsu N, Sakamoto M, Suzuki Y, Iwasaki M, Tamori A, Matsuura K, Kakinuma S, Sugauchi F, Sakamoto N, Nakagawa M, Yatsushashi H, Izumi N. 2012. Age and total ribavirin dose is an independent predictor of relapse among early virological responders to peg-interferon plus ribavirin therapy in chronic hepatitis C revealed by data mining analysis. *Antivir Ther* 17:35–43.
- Kwo PY, Lawitz EJ, McCone J, Schiff ER, Vierling JM, Pound D, Davis MN, Galati JS, Gordon SC, Ravendhran N, Rossaro L, Anderson FH, Jacobson IM, Rubin R, Koury K, Pedicone LD, Brass CA, Chaudhri E, Albrecht JK. 2010. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alpha-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): An open-label, randomised, multicentre phase 2 trial. *Lancet* 376:705–716.
- LeBlanc M, Crowley J. 1995. A review of tree-based prognostic models. *Cancer Treat Res* 75:113–124.
- Lleiter U, Buettner PG, Eigentler TK, Garbe C. 2004. Prognostic factors of thin cutaneous melanoma: An analysis of the Central Malignant Melanoma Registry of the German Dermatological Society. *J Clin Oncol* 22:3660–3667.
- Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. 2001. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: A randomised trial. *Lancet* 358:958–965.
- McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mak C, Garaud JJ, Albrecht JK. 2002. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 123:1061–1069.
- McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, Alam J, Muir AJ. 2009. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 360:1827–1838.
- Miyaki K, Takei I, Watanabe K, Nakashima H, Omae K. 2002. Novel statistical classification model of type 2 diabetes mellitus patients for tailor-made prevention using data mining algorithm. *J Epidemiol* 12:243–248.
- Ochi H, Maekawa T, Abe H, Hayashida Y, Nakano R, Kubo M, Tsunoda T, Hayes CN, Kumada H, Nakamura Y, Chayama K. 2010. Inosine triphosphate pyrophosphatase polymorphism affects ribavirin-induced anemia and outcomes of therapy—A genome-wide study of Japanese hepatitis C virus patients. *Gastroenterology* 139:1190–1197.
- Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battagay M, Bernasconi E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Gunthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Mullhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F, Telenti A, Bochud PY. 2010. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: A genome-wide association study. *Gastroenterology* 138:1338–1345.
- Sakamoto N, Tanaka Y, Nakagawa M, Yatsushashi H, Nishiguchi S, Enomoto N, Azuma S, Nishimura-Sakurai Y, Kakinuma S, Nishida N, Tokunaga K, Honda M, Ito K, Mizokami M, Watanabe M. 2010. ITPA gene variant protects against anemia induced by pegylated interferon-alpha and ribavirin therapy for Japanese patients with chronic hepatitis C. *Hepatology* 40:1063–1071.
- Seto WK, Tanaka Y, Liu K, Lai CL, Yuen MF. 2011. The effects of IL-28B and ITPA polymorphisms on treatment of hepatitis C virus genotype 6. *Am J Gastroenterol* 106:1007–1008.
- Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Muller T, Bahlo M, Stewart GJ, Booth DR, George J. 2009. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 41:1100–1104.
- Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. 2009. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 41:1105–1109.
- Tanaka Y, Kurosaki M, Nishida N, Sugiyama M, Matsuura K, Sakamoto N, Enomoto N, Yatsushashi H, Nishiguchi S, Hino K, Hige S, Itoh Y, Tanaka E, Mochida S, Honda M, Hiasa Y, Koike A, Sugauchi F, Kaneko S, Izumi N, Tokunaga K, Mizokami M. 2011. Genome-wide association study identified ITPA/DDRGGK1 variants reflecting thrombocytopenia in pegylated interferon and ribavirin therapy for chronic hepatitis C. *Hum Mol Genet* 20:3507–3516.
- Thompson AJ, Fellay J, Patel K, Tillmann HL, Naggie S, Ge D, Urban TJ, Shianna KV, Muir AJ, Fried MW, Afdhal NH, Goldstein DB, McHutchison JG. 2010a. Variants in the ITPA gene protect against ribavirin-induced hemolytic anemia and decrease the need for ribavirin dose reduction. *Gastroenterology* 139:1181–1189.
- Thompson AJ, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, Urban T, Afdhal NH, Jacobson IM, Esteban R, Poordad F,