**Table 2** Factors associated with sustained virological response to combination therapy

| Variable | Simple | | | Multiple | | | |
|---|---|---|---|---|---|---|---|
| | *n* | OR | *P* | *n* | OR | (95% CI) | *P* |
| Age | 840 | 0.393 | $3.16 \times 10^{-11}$*** | 517 | 0.386 | (0.27–0.56) | $5.08 \times 10^{-7}$*** |
| Sex (male vs. female) | 840 | 0.521 | $3.61 \times 10^{-6}$*** | 517 | 0.52 | (0.35–0.78) | 0.001459** |
| BMI (kg/m$^2$) | 834 | 0.8 | 0.1094 | | | | |
| Viral load (Log IU/ml) | 840 | 0.761 | 0.001828** | | | | |
| Core aa70 substitution | 840 | 0.537 | $1.98 \times 10^{-5}$*** | 517 | 0.507 | (0.35–0.74) | 0.000521*** |
| Core aa91 substitution | 840 | 0.818 | 0.1568 | | | | |
| ISDR (0–1 vs. ≥2) | 840 | 2.36 | $5.19 \times 10^{-5}$*** | 517 | 2.12 | (1.19–3.77) | 0.01037* |
| Hypertension | 651 | 0.625 | 0.02389* | | | | |
| Diabetes | 681 | 0.794 | 0.4464 | | | | |
| Blood transfusion | 732 | 1 | 0.9788 | | | | |
| Fibrosis (F0–1 vs. F2–4) | 732 | 0.674 | 0.008287** | | | | |
| Activity (A0–1 vs. A2–4) | 725 | 0.779 | 0.09567 | | | | |
| Steatosis | 502 | 0.645 | 0.03413* | | | | |
| Prior IFN treatment | 830 | 1.37 | 0.02648* | | | | |
| HDL cholesterol (mg/dl) | 493 | 0.761 | 0.1333 | | | | |
| LDL cholesterol (mg/dl) | 529 | 1.46 | 0.03223* | 517 | 1.61 | (1.10–2.38) | 0.01521* |
| Triglyceride (mg/dl) | 726 | 0.913 | 0.5412 | | | | |
| Total cholesterol (mg/dl) | 814 | 1.25 | 0.11 | | | | |
| AST (IU/l) | 783 | 0.933 | 0.6316 | | | | |
| ALT (IU/l) | 840 | 0.972 | 0.837 | | | | |
| WBC (/mm$^3$) | 836 | 1.55 | 0.001831** | | | | |
| Hemoglobin (g/dl) | 838 | 1.34 | 0.00276** | | | | |
| Platelets ($\times 10^4$/mm$^3$) | 838 | 1.74 | $7.92 \times 10^{-5}$*** | | | | |
| Gamma-GTP (IU/l) | 823 | 0.735 | 0.0288* | 517 | 0.656 | (0.43–0.99) | 0.04588* |
| Albumin (g/dl) | 809 | 1.41 | 0.01699* | | | | |
| Ferritin (μg/l) | 532 | 0.898 | 0.5404 | | | | |
| Treatment period (weeks) | 840 | 1.02 | 0.6095 | | | | |

Simple and multiple logistic regression was used to examine the association between SVR and patient and viral factors. Factors with $P < 0.05$ were considered for inclusion in the multiple regression model and the best model selected by backwards stepwise selection using AIC

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

*IFN* interferon, *OR* odds ratio, *CI* confidence interval, *AIC* akaike information criterion

of treatment included age, sex, viral load, core aa70, LDL, platelets, and white blood cell counts, whereas for 72 weeks of treatment only age, ISDR, and prior IFN treatment were significant, although LDL cholesterol was marginally significant (Table 3).

Among patients who underwent 48 weeks of therapy, 61% of patients with core aa 70 wild-type achieved an SVR compared to only 44% of patients with mutant core aa 70 ($P = 1.8 \times 10^{-5}$, Fig. 1a), whereas for 72-week patients, the ratio was 1:1 (Fig. 3a). Conversely, in the 48-week group, 71% of patients with two or more mutations in the ISDR were able to achieve an SVR compared to 52% with the wild-type ISDR, and in the 72-week group (Fig. 1b), 80% of patients with two or more ISDR mutations achieved an SVR compared to 54% with zero or one ISDR mutations (Fig. 3b). Median baseline viral load was significantly lower in 48-week SVR patients compared to that in non-SVR patients ($P = 0.001$, Fig. 1c), whereas there was no significant difference between viral load and SVR in 72-week therapy patients ($P = 0.625$, Fig. 4c). There was a significant effect of age and treatment outcome among 48-week patients ($P = 9.3 \times 10^{-6}$, Fig. 2), but the difference was not significant among 72-week therapy patients. However, the proportion of patients achieving an SVR tended to decrease with age in both groups, particularly in females over age 70 years in the 72-week group (Figs. 2, 4).

**Table 3** Independent factors associated with sustained virological response to 48- and 72-week peg-interferon plus ribavirin combination therapy

| Variable | 48 Weeks | | | 72 Weeks | | | |
|---|---|---|---|---|---|---|---|
| | *n* | OR | *P* | *n* | OR | (95% CI) | *P* |
| Age | 535 | 0.642 | 0.0165* | 133 | 0.4 | (0.176–0.91) | 0.02877* |
| Sex (male vs. female) | 535 | 0.481 | 0.000284** | | | | |
| Viral load (Log IU/ml) | 535 | 0.738 | 0.01033* | | | | |
| Core aa70 substitution | 535 | 0.454 | $9.95 \times 10^{-5}$** | | | | |
| ISDR (0–1 vs. ≥2) | 535 | 2.75 | 0.000358** | 133 | 7 | (1.35–36.2) | 0.02047* |
| Fibrosis (F0–1 vs. F2–4) | 535 | 0.66 | 0.03954* | | | | |
| Prior IFN treatment | | | | 133 | 2.67 | (1.22–5.85) | 0.01431* |
| LDL cholesterol (mg/dl) | | | | 133 | 2.04 | (0.952–4.35) | 0.06673 |
| WBC ($/mm^3$) | 535 | 1.53 | 0.03342* | | | | |
| Platelets ($\times 10^4/mm^3$) | 535 | 1.54 | 0.03707* | | | | |

Simple and multiple logistic regression analysis was used to examine the association between SVR and patient/viral factors separately for patients receiving 48 and 72 weeks of treatment

** $P < 0.001$, * $P < 0.05$

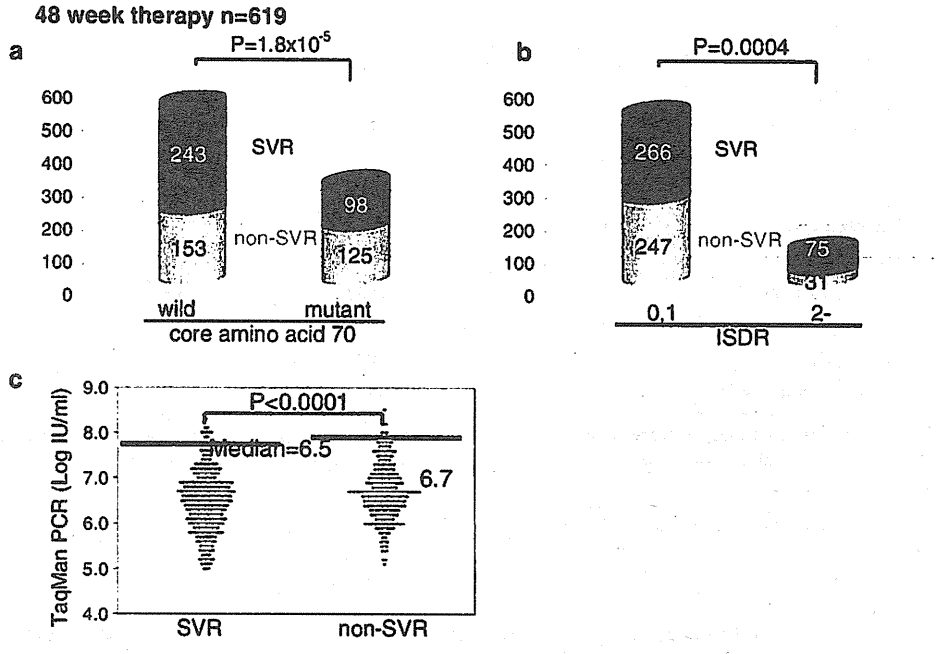
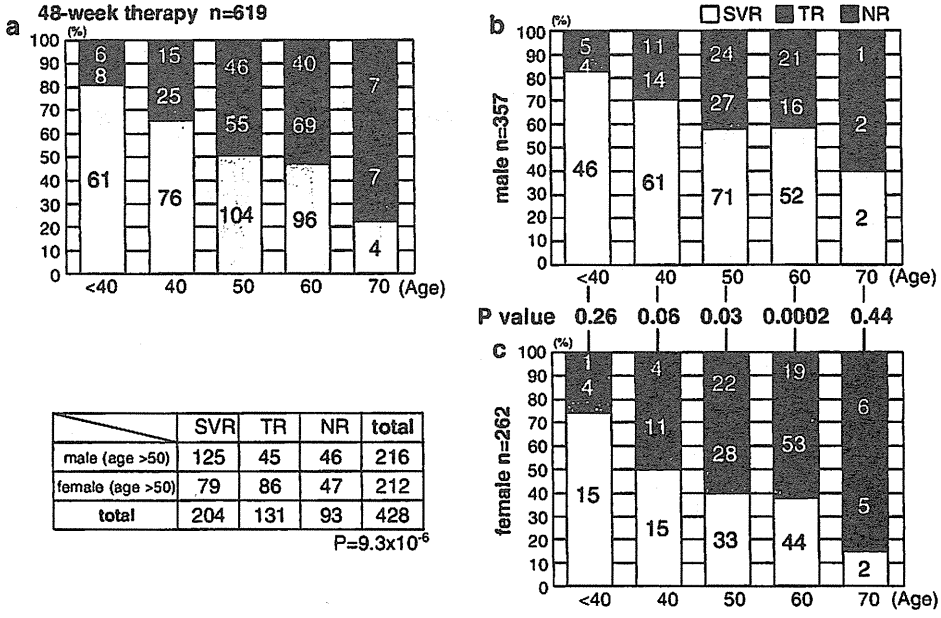



**Fig. 1** Viral factors for 48-week treatment. Relationships between sustained virological response (*SVR*) and a core amino acid 70 substitutions, b amino acid substitutions in the interferon sensitivity determining region, and c baseline viral titers grouped by SVR and non-SVR for patients treated for 48 weeks. *PCR* Polymerase chain reaction

## CART analysis

Figure 5 shows the decision tree generated by CART analysis. All variables were included during model construction, and the SimpleCart algorithm generated a tree based on the following fields: age, cholesterol, sex, γGTP, 48 versus 72 weeks of treatment, and aa substitutions in the ISDR and at core aa70. Age was used as the first cutoff, and patients younger than 46.5 years were classified as having a high probability for SVR (78%). Total cholesterol was identified as the next decision point, and patients with cholesterol higher than 211.5 mg/dl were classified as SVR if they were younger than 62.5 years (84%) and NR (65%) otherwise. Patients with cholesterol lower than 211.5 mg/dl were subdivided next by sex. Females who received 48 weeks of treatment were classified as NR (71%), whereas females receiving 72 weeks of treatment were classified as SVR if they were younger than 58.5 years (71%) or NR otherwise (64%). Males who were infected with aa70 wild-type were classified as SVR (62%), whereas males with aa70 substitutions were classified as NR if total cholesterol was less than 130 mg/dl (97%). Males with ISDR substitutions were classified as SVR (75%), and those with wild-type ISDR were classified




| | SVR | TR | NR | total |
|---|---|---|---|---|
| male (age >50) | 125 | 45 | 46 | 216 |
| female (age >50) | 79 | 86 | 47 | 212 |
| total | 204 | 131 | 93 | 428 |

$P=9.3\times10^{-6}$

**Fig. 2** Relationship between age and response to treatment for 48-week therapy. Treatment outcomes by age in 10-year intervals are shown for a all patients, b males only, and c females only. *NR* non-viral response

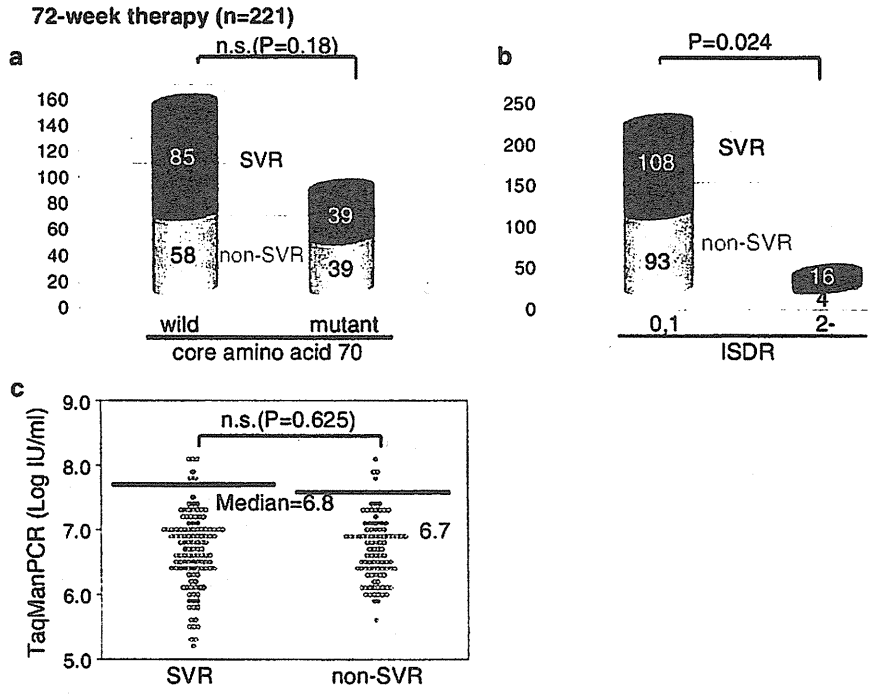
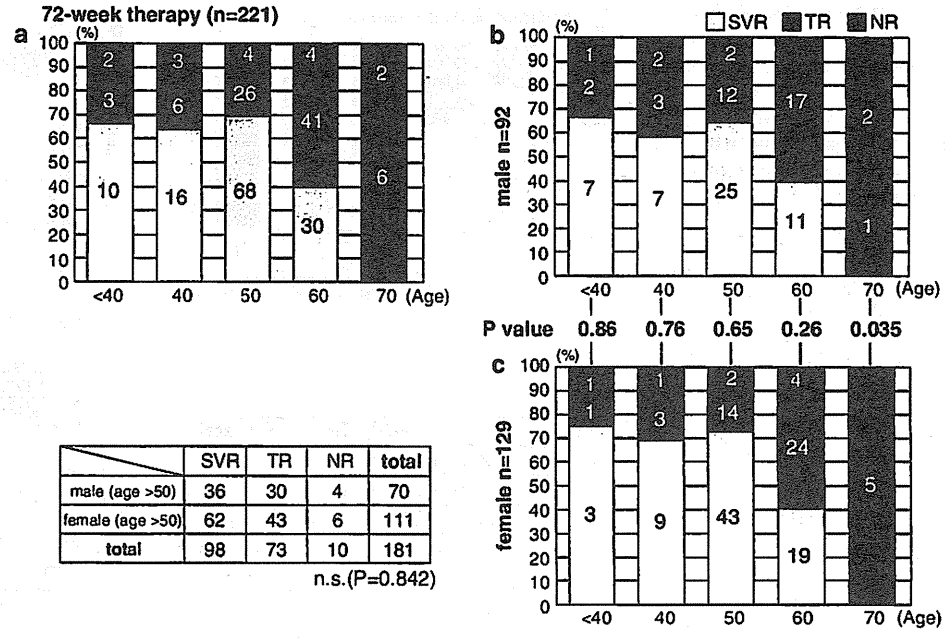



**Fig. 3** Viral factors for 72-week treatment. Relationships between sustained virological response and a core amino acid 70 substitutions, b amino acid substitutions in the interferon sensitivity determining region, and c baseline viral titers grouped by SVR and non-SVR for patients treated for 72 weeks. *n.s.*, Not significant

as SVR if γGTP was less than 48.5 IU/l (57%) and NR otherwise (77%).

All factors selected during tree construction were found to be significant in univariate analysis, except for treatment length and cholesterol, and each remained significant in multivariate logistic regression. Although LDL was included in the multivariate logistic model, it was not selected during tree construction. Tenfold cross-validation resulted in 65.2% correctly classified instances with a kappa statistic of 0.29. The true positive rate was 69.2%, the false positive rate was 39.7%, and precision was 68.4%.

To compare the performance of SVR prediction between the logistic and CART models, the WEKA Logistic classifier was used to perform tenfold validation based on the




| | SVR | TR | NR | total |
|---|---|---|---|---|
| male (age >50) | 36 | 30 | 4 | 70 |
| female (age >50) | 62 | 43 | 6 | 111 |
| total | 98 | 73 | 10 | 181 |

n.s.(P=0.842)

**Fig. 4** Relationship between age and response to treatment for 72-week therapy. Treatment outcomes by age in 10-year intervals are shown for a all patients, b males only, and c females only

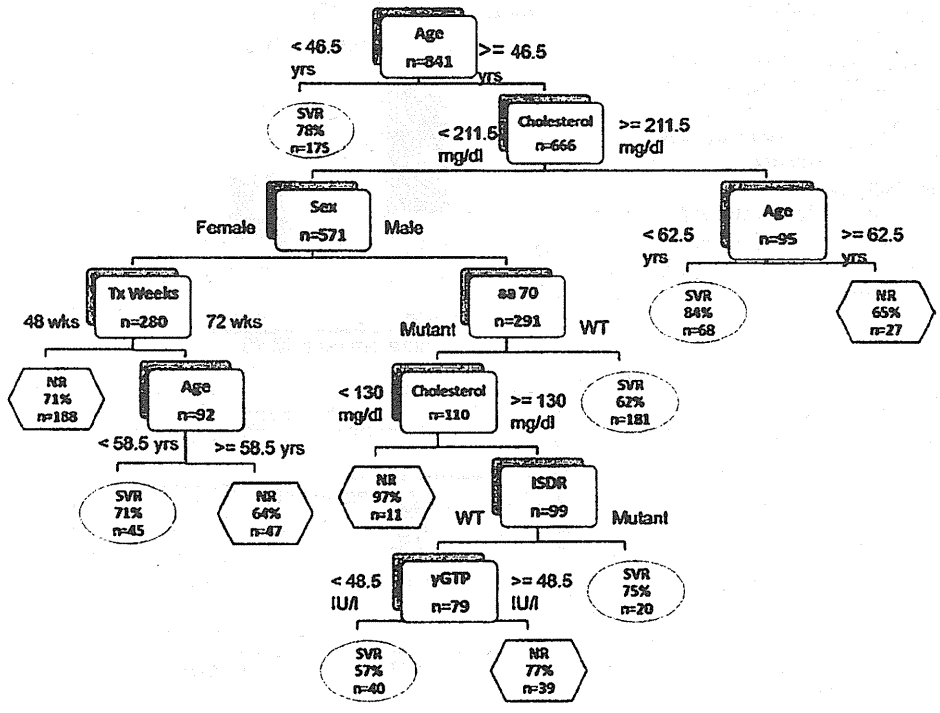



**Fig. 5** Decision tree for SVR prediction. *Boxes* represent branch points based on cutoff values for factors determined by the tree generation algorithm. Each branch contains two choices, and each path ends in a prediction for either SVR or NR with an associated probability. *yrs* Years, *Tx* treatment, *ISDR* interferon sensitivity determining region, *aa* amino acid, *WT* wild-type, *γGTP* γ-glutamyl transpeptidase

multivariate logistic regression model above. The true positive rate for the logistic classifier was somewhat higher, at 73.1%, but with a slightly worse false-positive rate of 48%, and 63.7% correctly classified instances with a kappa statistic of 0.25 and precision 0.65. Receiver operating characteristic (ROC) curves were very similar, and the area under the curve was 0.677 for the CART model and 0.696 for the logistic model.

## Discussion

Using two complementary approaches we identified several pretreatment factors predictive for SVR in patients treated for 48 and 72 weeks. Logistic regression and CART analysis both suggest that sex, age, cholesterol, and substitutions at core aa70 and ISDR are associated with SVR in patients with a high viral load of genotype 1b. Based on

the decision tree topology and a significant interaction between sex and treatment duration, it appears that 72 weeks of treatment may be most beneficial in women between the ages of 46 and 58 years who have low cholesterol. In general, patients who are younger, male, have cholesterol over 130 mg/dl, or who have wild-type core aa70 or mutant ISDR are the most likely to achieve an SVR.

Because each of the above values can be determined prior to treatment and are interpretable by clinicians, they may be useful as a guide when establishing a treatment regimen in the case of potentially difficult-to-treat patients. Once IFN treatment has been started, early and/or rapid viral response is likely to be the strongest predictor of SVR [33], and slow responders have been shown to be the most likely to benefit from extended treatment [34, 35]. However, because of the expense, low success rate, and potential side effects of IFN-based therapy, predictors available prior to treatment are also needed. Factors predictive of NR may help guide the decision to avoid or discontinue IFN therapy in patients with a low probability of SVR, and factors predictive of SVR may help identify subsets of patients who are likely to achieve an SVR if treated longer than the standard 48-week regimen.

Several other recent studies have examined predictors for SVR for 72 weeks of treatment, although nearly all focus on on-treatment predictors and conclude that 72-week therapy significantly improves SVR rates in slow responders [9, 10, 35]. Ferenci et al. [11] also showed that extension to 72 weeks decreased the relapse rate among early viral responders. In a large retrospective cohort study, Watanabe et al. [36] dissected a complex relationship between SVR and age, sex, and viral load similar to that reported here, although results are difficult to compare because they did not measure cholesterol or viral substitutions. While they recommend 72-week therapy for all slow-responding patients regardless of sex or age, they note that the SVR rate was surprisingly high among elderly female patients following 72-week treatment, noting that the SVR for 48-week treatment was typically low among older female patients in Japan, which they suggest could be related to the development of insulin resistance associated with menopause [36]. Other studies discourage the use of 72-week therapy for all patients except in the specific case of slow responders [8]. Moreover, in a large prospective study, Buti et al. [34] conclude that 48-week combination therapy should remain the standard of care even for slow responders, due to the increased cost and incidence of adverse events relative to a modest increase in the SVR rate. They clarify, however, that patients with a less than 2 log decline at week 8 and undetectable HCV RNA at week 24 are the most likely to benefit from 72-week treatment. Unfortunately they did not examine other predictors in a multivariate analysis. Because each of these studies hinges on rapid versus slow viral response and an on-treatment predictor requiring up to 24 weeks of treatment to establish, pretreatment predictors of early viral kinetics, including those presented here (e.g., viral substitutions and baseline cholesterol levels [12]), may be useful for predicting the outcome of extended therapy prior to treatment [17].

The combination of multiple approaches to identify predictive factors should help improve confidence in the results and partially protect against the bias inherent in any single approach. Comparing the results of a standard analysis with an alternative technique may reveal which variables are robust and which are sensitive to methodological differences. There are many different classification tools, including neural networks, Bayesian networks, and support vector machines, but models based on these may be more difficult to interpret or apply in clinical practice. On the other hand, decision tree approaches such as C4.5 and CART are widely used in biomedical studies [37–39] and provide a simple and intuitive hierarchical format that in many cases can be used without a computer.

The lack of randomized assignment of patients to duration of treatment limits the conclusions that can be drawn from the present study, and additional predictive factors, particularly interleukin (IL) 28B single-nucleotide polymorphism (SNP) genotype and viral kinetics, should be included in future prospective studies. Comparison of ROC curves suggests that the performance of the two models in the present study is similar, although neither is sufficiently sensitive or specific for accurate clinical prediction based on the number of patients analyzed. Nonetheless the strong overlap between the variables selected by each method suggests that several patient factors, including age, sex, and cholesterol level, as well as several viral factors, including core aa70 and ISDR substitutions, are robust predictors for SVR. Differences in the variables selected between the two approaches suggest that several models with similar predictive ability are also possible. In the regression model, LDL cholesterol but not total cholesterol was an independent factor associated with SVR, whereas in the CART analysis total cholesterol was selected instead. This may be due to the hierarchical nature of decision tree models, which may yield better results in the face of missing data, higher-order interactions, or nonlinear relationships. Comparison of separate models for 48 and 72 weeks also suggests that age and ISDR substitutions are important predictors of SVR for patients undergoing 72 weeks of treatment, whereas the decision tree suggests that the 72-week treatment length is important mainly for a subgroup of female patients. Without greater understanding of the role of HCV core and ISDR substitutions, it is difficult to interpret the role of these predictors, as well as

potential interactions with cholesterol level and other clinical factors. Further studies should be performed to investigate these interactions and to better characterize the subgroup of patients who are most likely to respond to long-term IFN therapy.

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**Conflict of interest** None of the authors have conflicts of interest to declare.

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# 第61回　名古屋市立大学医学会総会
## 特　別　講　演　Ⅱ

# IL28B：C型肝炎テーラーメイド治療の幕開け

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IL28B: Drive the hepatitis C treatment setting toward a tailored approach

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## はじめに

C型肝炎ウイルス（HCV）感染者数は，現在，全世界で1億7000万人，わが国で約200万人の感染者がいると推定されている．一般にHCVは感染が成立すると70～80%は持続感染を呈し，慢性肝炎から肝硬変，さらに肝硬変から高率に肝がんへと移行する．したがって，C型慢性肝炎に対する抗ウイルス療法は重要であり，現行の標準治療であるペグインターフェロン＋リバビリン（PEG-IFN/RBV）併用療法でウイルス排除が可能となったが，日本人に最も多い遺伝子型1型高ウイルス量の症例では50%程度の根治しか得られず，約15～20%はPEG-IFN/RBV併用療法が全く効かないのが現状である．今年度に新たなC型肝炎ウイルス蛋白を標的とした治療薬が登場し，治療成績の向上が期待されるが，わが国では高齢者の割合が多いため，副作用発現頻度が高まる可能性があり，より適応症例を選別することが要求される．従来から治療効果予測因子として，様々なウイルス側因子，宿主側因子が知られているが，2009年に報告されたIL28B遺伝子多型を中心に標準的なPEG-IFN/RBV併用療法の現状を述べる．

## 従来の治療前効果予測因子

現行のHCVに対する薬物治療は，その治療期間は1年から1年半にわたり，副作用も高頻度に発現するため，治療費や身体的な負担が大きい治療である．したがって，もし治療前に治療効果や随伴する有害事象を予測する事ができれば，医療費の軽減と患者の負担を軽減することが可能となるため，様々な治療効果を予測する因子の探索が行われてきた．IFN療法における治療前予測因子としては，ウイルス側の因子，薬剤による因子，宿主側の因子が挙げられる（図1）．

ウィルス因子（表1）

PEG-IFN/RBV併用療法における治療効果予


図1　IFNを用いたC型肝炎治療効果を規定する各種因子

表1　現在のIFN治療効果に寄与するウイルス因子

| ウイルス因子 | 変異 | IFN治療効果 |
|---|---|---|
| HCV genotype | Genotype 1 | 抵抗性 |
| | Genotype 2 | 反応性 |
| Core 70 | R→Q, H | 抵抗性 |
| Core 91 | L→M | 抵抗性 |
| ISDR | 0 | 抵抗性 |
| | 1-3 | 中間 |
| | 4≦ | 反応性 |
| IRRDR | 6> | 抵抗性 |
| | 6≦ | 反応性 |

測ウイルス因子として，これまでにHCV遺伝子型(genotype)，ウイルス量，コア領域やNS5A領域のアミノ酸変異などが報告されている．一般に，HCV遺伝子型については，薬物治療に対する反応性が乏しい1型と，治療反応性の高い2型に分類される．

次に，治療効果を高めるウイルス変異として，HCV遺伝子の非構造領域であるNS5Aの後半部に存在する40アミノ酸程度の領域に多様性があることが知られている．この領域は，インターフェロン感受性決定領域（ISDR）と定義され，この領域での変異の数が治療反応性と相関することが知られている[1]．通常は，変異がない野生型，1～3個の変異を有する中間型，4個以上の変異を有する変異型に分類され，IFN単独24週投与でのウイルス陰性率はISDR野生型では10%未満であり，治療効果とISDRの高い相関が認められた．しかし，現在はPEG-IFN/RBV併用療法が標準治療となり，ISDRに代わってHCVのNS5A内でV3領域にかかるaa2334-2379領域(interferon/ribavirin resistance-determining region: IRRDR）の変異数と治療効果との関連性が報告された[2]．

その他によく知られるウイルス因子として，HCVコア領域の70番，91番の変異がある．治療反応例と不応例を比較すると，コア領域の70番，91番の変異が治療不応例において観察され，多変量解析を行うとこれらの変異が治療不応に強い関連を示すことが報告された[3]．

薬剤側因子

治療効果に関連する薬剤因子としては，投薬期間と服薬量が挙げられる．現在はウイルス陰性化時期に応じて投薬期間を48週投与あるいは72週まで延長する工夫など治療効果の向上が試みられている．また，予定服薬量の80%以上が投与された群においてその治療効果が高いことなどが知られている．しかし，これらはいずれも治療開始後の個々の症例の反応性を基に判断可能な因子であったが，近年では宿主因子であるITPAが服薬率に関連する治療前因子として挙げられる．

宿主側因子

従来，PEG-IFN/RBV併用療法における治療効果予測の宿主側因子として，年齢，性差，肝線維化進展度，インシュリン抵抗性など多数報告されていたが，これらの因子と前述したウイルス側因子を総動員して解析しても，治療前の効果予測は約50%程度に留まることが知られていた．一方，ヒトゲノム計画の成功により，ヒト遺伝子は個人差として約300個に1個，全ゲノムで約1,000万カ所の一塩基多型（SNP：single nucleotide polymorphism）が存在し，このSNPが個々の疾患の発症，薬剤反応性や副作用に大きく関与することが続々と明らかとなってきている．近年，ゲノムワイドに均一に配置された約90万箇所（日本人では62万箇所）のSNPsを一括タイピングすることが可能となり，この手法を用いて我々を含めた世界中から，C型慢性肝炎のIFN治療反応性に極めて強く関連するSNP同定の報告がなされた[4-6]．

## IL28B遺伝子多型と治療効果

我々は，PEG-IFN/RBV併用療法の有効性に関連するSNPを同定するために，ゲノムワイド関連解析（GWAS：genome-wide association study）を実施した．すなわち，PEG-IFN/RBV併用療法が有効（再燃例も含む）であった日本人患者と無効であった患者142人に関して，ヒト遺伝子の中で個人差があるとされる約90万箇所をAffymetrix Genome-Wide Human SNP

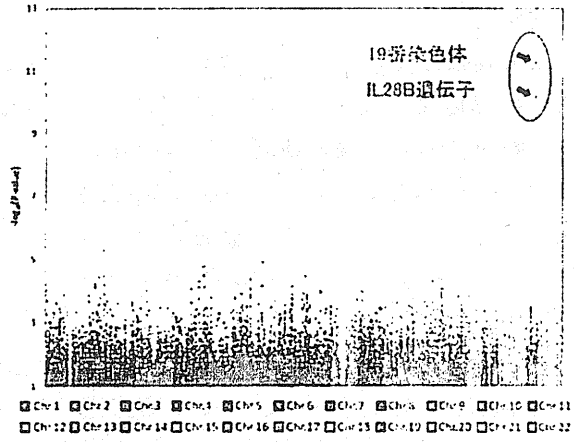


図2　ゲノムワイド関連解析
PEG-IFN/RBV 併用療法が有効（再燃例も含む）例と無効例で各染色体上のそれぞれのSNPのアリル頻度について比較し算出したP値のプロット．19番染色体のIL28B遺伝子周辺に治療無効に関連する有意なSNPsを発見した．

Array6.0を用い分析した結果，19番染色体のIL28B遺伝子周辺に治療無効に関連する有意なSNPsを発見した（図2）．別のコホート（検証群172人）を用いて，IL28B遺伝子及び遺伝子周辺を詳細に検討した結果，治療反応性に強く関与するSNPsは複数存在し，しかもすべてが連鎖不平衡（複数の遺伝子座の遺伝的多型の間にランダムでない相関がみられること）であった．このうち代表的なSNPであるrs8099917（マイナーアレルG）の治療効果別のアレル頻度を解析すると，治療反応群（著効群＋再燃群）では，メジャーアレル（TT）の割合が高く，無効群ではマイナーアレル（TG＋GG）の割合が高く，マイナーアレルの患者では治療抵抗性であることが示された．さらに，従来からインターフェロン治療効果に寄与する因子であることが報告されている年齢，性別，血小板数，肝線維化，HCV-RNA量を含めて多変量解析を行ったところ，これらの因子をはるかに凌ぐ危険率約30倍の確率（$P=2.68\times10^{-32}$）で，rs8099917のSNPがPEG-IFN/RBV併用療法の無効に寄与していることが明らかとなった[6]．

一方，PEG-IFN/RBV併用療法の有効性に関連するGWASについて，同時期に欧米でも報告があり[4,5]，いずれの報告もIL28B遺伝子多型が関与する結果であった（表2）．Geらの報告では，白人（871人），黒人（191人），ヒスパニック（75人）で検討した結果，白人においてIL28B遺伝子から3kb上流のSNP（rs12979860）が著効に強く関連することがわかった．興味深いことに，rs12979860のメジャーアレル（C-アレル）の頻度は，アジアで最も多く（80–90%），続いて白人（European-Americans）及びヒスパニック（Hispanics）が70–80%，そして黒人（African-Americans）は30–50%と低値であり，以前より指摘されているアフリカ系で治療反応性が悪いことを遺伝的に説明するものであった[1]．一方，Suppiah Vらは，我々と全く同じSNPであるrs8099917が最も有意なSNPsとして報告している[5]．rs12979860とrs8099917は一見異なる結果であるようにみえるが，これらのSNPsはゲノム上の近傍した位置にあり，HapMapデータによると連鎖不平衡が成立していると考えられ（図3），実際に日本人では99%一致した結果となり（表3），解析に使用したプラットフォームの違いと考えられる（表2）．

表2　GWAS結果に基づいたC型肝炎治療効果に関連するIL28B周辺SNP報告内容

| Study | Ge et al. (4) | Suppiah et al. (5) | Tanaka et al. (6) |
|---|---|---|---|
| Region | Northern America | Northern Europe Australia | Japan |
| Ancestry | Caucasian/ African /Hispanic | Caucasian | Japanese |
| GWAS size | 871/ 191/ 75 | 293 | 142 |
| Replication | No replication | 555 | 172 |
| Case/ control | SVR vs. non-SVR | SVR vs. non-SVR | VR vs. NVR<br>SVR vs. non-SVR |
| Adherence | Over 80% adherent to PEG-IFN/RBV during the first 12 weeks of therapy | Not controlled | Over 80% adherent to PEG-IFN/RBV during the first 12 weeks of therapy |
| Significant SNPs | rs12979860 | rs8099917 | rs8099917 |
| P value | $1.37\times10^{-28}$ | $9.25\times10^{-9}$ | $1.18\times10^{-14}$ |
| OR (SVR) | 3.1 (2.1-4.7) | 1.98 (1.57-2.52) | 12.1 (6.5-22.4) |
| Platform | Illumina 610-quad | Illumina CNV370-quad | Affymetrix SNP 6.0 |

## IFNλの抗ウイルス効果

IL28Bは19番染色体長腕に位置し約1.5kと非常に小さく，IFNλ3をコードするが，これは通常のC型肝炎の治療に使用されているIFNαやβとは異なるIFNλの1種である．IFNλには，1，2，3が存在しそれぞれ

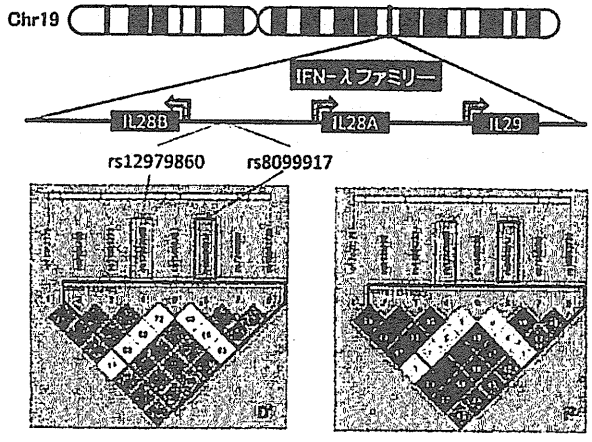


図3　IFN-λファミリーの遺伝子構造と治療効果に寄与するSNPsのHapMapデータ

表3　PEG-IFN/RBV併用療法の有効性に関連すると見出された各SNPsの日本人に対する関連性

| | NVR (n=74) | | | VR (n=160) | | | | |
|---|---|---|---|---|---|---|---|---|
| | AA | AB | BB | AA | AB | BB | *P-value* | OR |
| rs12980275 | 13 | 58 | 3 | 135 | 25 | 0 | $6.42\times10^{-23}$ | 25.3 |
| rs11881222 | 13 | 59 | 2 | 142 | 18 | 0 | $9.41\times10^{-27}$ | 37.0 |
| rs12979860 | 13 | 59 | 2 | 141 | 19 | 0 | $3.66\times10^{-26}$ | 34.8 |
| rs8099917 | 13 | 59 | 2 | 142 | 18 | 0 | $9.41\times10^{-27}$ | 37.0 |

日本人のC型慢性肝炎234人に対するIL28B遺伝子周辺に存在する代表的SNPsのリスクハプロタイプ一覧を示した．日本人ではrs8099917とrs11881222は100%連鎖不平衡が成立し，rs12979860に関してもrs8099917と99%連鎖不平衡が成立していることがわかる．

IL29，IL28A，IL28Bがコードする．IFNλは，レセプターは異なるがIFNα/βと同じシグナル伝達系であるJAK/STAT経路を活性化し，その下流に存在するIFN誘導遺伝子群（ISGs：interferon-stimulated genes）を誘導して抗ウイルス効果をもたらすことが報告されている（図4）．しかし，実際にC型肝炎患者においてIL28B遺伝子すなわちIFNλがどのような機序でIFN治療（特にIFNα治療）効果に影響するかは未だ不明である．

これまでにC型肝炎感染患者におけるIL28B遺伝子発現に関していくつかの報告がなされた．末梢血単核球を用いた発現解析では，IL28Bマイナーアレル群でIL28遺伝子発現レベルが低く[5,6]，一方，肝臓内に関しては，IL28Bマイナーアレルを有する治療抵抗群において，治療前の肝臓内ISGの発現レベルが高いことが報告された[7,8]．従来から報告されていた，治療前ISG発現が高いグループではIFN-αによるISG誘導が弱く，抗ウイルス効果が期待できない[9,10]ことをIL28B遺伝子型で説明する内容であった．

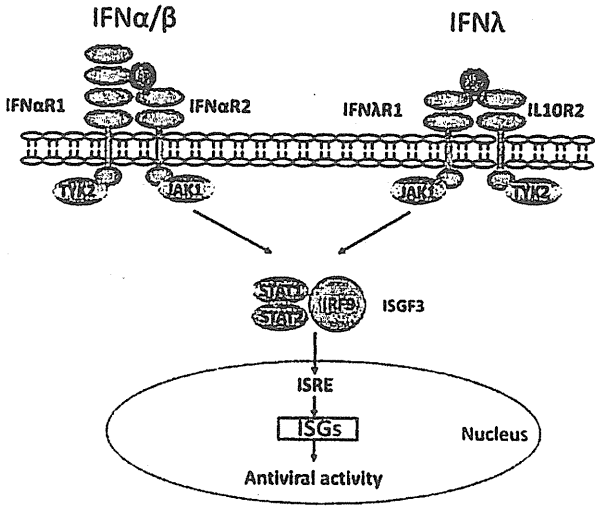


図4　インターフェロンシグナル伝達
INFλはIFNα/βとレセプターは異なるが，同じJAK/STAT経路を活性化し，その下流に存在するIFN誘導遺伝子群（ISGs）を誘導して抗ウイルス効果をもたらす．

このようにIL28B遺伝子型による機能的差異がC型肝炎治療効果に影響する事が示唆される中，現在アメリカで慢性C型肝炎患者に対するIFNλ1の臨床試験（現在phase Ⅱが進行中）が既に実施され，副作用が少なくその有効性が期待されている[11]．

## C型急性肝炎時におけるIL28B遺伝子多型の関与

一方，IL28B遺伝子多型はHCVの自然排除にも関連することが明らかとなった．すなわち，HCVを自然に排除した患者388例と持続感染が成立した患者620例のコホート研究において，治療感受性であるrs12979860の遺伝子型（CC）では，HCVの自然排除を促進することが報告された[12]．その後，異なるコホート研究において，rs8099917関しても同様の内容が報

告された[13]．さらに興味深いことに，オーストラリアのグループから，治療感受性であるrs 8099917のメジャーアレル（TT）の自然排除率が高いことに加え，HCV自然排除の予測因子として肝炎発症時の黄疸合併が挙げられ，さらにこの黄疸合併頻度がメジャーアレル（TT）群で有意に高いことが報告された[14]．このことは，ウイルス感染細胞排除に作用する細胞性免疫応答にもIL28B遺伝子多型が影響する事を示唆するものである．

## おわりに

現在，先進医療として厚生労働省から認められた「IL28Bの遺伝子診断によるインターフェロン治療効果の予測評価」により，IL28B遺伝子多型検査が特定の施設で可能となり，これまでにない高い確率で治療効果を予測することができるようになった（約80%の的中率）．治療効果予測のさらなる的中率向上が必要であるものの，この因子の同定によりテーラーメイド医療が現実のものとなりつつある．すなわち，治療無効と予測された場合，現状のPEG-IFN/RBV併用療法をあえて行わない選択肢もあり，新規治療法や少量長期IFN治療（発癌抑止目的）を選択することで無用な副作用の苦痛や出費から免れることができる（図5）．

一方で，今年度新たに登場するプロテアーゼ阻害剤（テラプレビル）を追加した3剤併用療法でも，IL28B遺伝子型は治療効果予測因子として重要であることが報告され[15]，近い将来に続々と登場してくる直接的な抗ウイルス剤とペグインターフェロンとの併用療法による治療効果もIL28Bを中心とした宿主因子の応答により影響されることが示唆される．

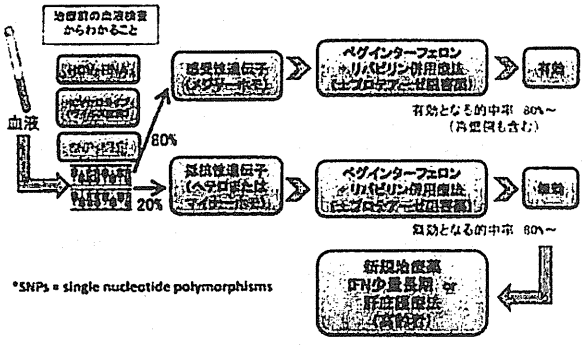


図5　IL28B遺伝子多型検査に基づいた治療戦略
セログループ1型／高ウイルス量の患者（C型慢性肝炎患者全体の約60%）を対象として，IL28B（IFN-λ）領域遺伝子多型（SNPs）を治療前に測定することによりPEG-IFN/RBV治療効果を高い確率で予測可能である．

今回検出された宿主因子（IL28B）は先天的な要因であるが，今後は後天的なゲノム変化やこれまでのウイルス因子も考慮に入れる必要がある．それらの複合的な解析により，治療効果を左右する真のウイルス側，宿主側の因子を同定し，テーラーメイド医療を実現するために，さらなる予測的中率の向上を計っていくことが必要である．

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# Development of new *IL28B* genotyping method using Invader Plus assay

Running title: Development of *IL28B* genotyping assay

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*Abbreviations: GWAS, genome-wide association study; HCV, hepatitis C virus; NPV, negative predictive value; NVR, null virological response; PEG-IFN-α, pegylated interferon alpha; PPV, positive predictive value; RBV, ribavirin; SVR, sustained virological response; TVR, transient virological response.*

**Abstract**

*IL28B* polymorphism is associated with the response to pegylated-interferon alpha with ribavirin (PEG-IFN-α/RBV) treatment in chronic hepatitis C patients. As a genotyping assay for *IL28B* SNPs in clinical practice, the Invader Plus assay was developed. The accuracy, intra-assay, inter-assay precision, and the limit of detection of the Invader Plus assay were evaluated. Two SNPs (rs8099917 and rs12979860) associated with *IL28B* were genotyped by the Invader Plus and TaqMan assay in 512 Japanese patients. In comparison with direct sequencing, the Invader Plus assay showed 99% accuracy in rs8099917 and 100% accuracy in rs12979860. Intra-assay and inter-assay precision were sufficient to use in clinical practice and the detection limit was 1ngDNA/assay. Genotyping by rs8099917 showed that 361 (71%), 144 (28%) and 7 (1%) of the patients were major homozygous, heterozygous and minor homozygous types, respectively. Five of the 512 cases (1%) had haplotype differences, but none showed differences between the two genotyping methods. For patients with HCV genotype 1, the prevalence of responders in the major homozygous type was 83.3%, and that of non-responders in the minor heterozygous/homozygous type was 72.5%. A convenient *IL28B* genotyping method using the Invader Plus assay could be useful to predict the treatment outcome in clinical practice.

*Keywords:* Interferon, *IL28B*, SNP genotyping, Invader Plus assay

## Introduction

Hepatitis C virus (HCV) infection results in cirrhosis and hepatocellularcarcinoma worldwide (1). Pegylated interferon alpha with ribavirin (PEG-IFN-α/RBV) is currently the most dominant therapy for chronic HCV infection, but roughly 50% of patients with genotype 1b, the most common in Japan, are not able to achieve a sustained virological response (SVR) determined by the serum HCV-RNA level 24 weeks after treatment (2, 3). In addition, this therapy often leads to side effects, such as flu-like symptoms, depression and anemia(4); therefore, it is valuable to predict the particular response before treatment with PEG-IFN-α/RBV to avoid these side-effects and to avoid ineffective therapy.

Not only viral factors (genotype and viral mutation), but also host factors influence the therapeutic outcome. Age, sex, body mass index and histological grade are considered to determine the individual's treatment regimen and outcome (5, 6, 7). Recently, it has been reported through a genome-wide association study (GWAS) of patients with genotype 1 HCV that single nucleotide polymorphisms (SNPs) located near the *IL28B* gene are strongly associated with a response to PEG-IFN-α/RBV therapy in Japanese (8), European (9), and a multi-ethnic population (10, 11). In particular, the two outstanding SNPs, rs12979860 and rs8099917 (located ~3 kb and 8 kb upstream of *IL28B*, respectively) have been found in strong association with the treatment response (8, 10). The minor allele frequency of rs8099917 was significantly higher in the null virological response (NVR) group than the virological response group. By taking advantage of *IL28B* typing, it may be possible to predict a NVR as well as a SVR in order to tailor the most suitable treatment regimens.

In this study, the "Invader Plus genotyping assay *IL28B* SNP" test kit including primers and probe setting was developed and compared with the usual TaqMan probe assay for genotyping *IL28B* SNPs and the pre-treatment prediction of the response to

PEG-IFN-α/RBV therapy in HCV infection.

## Materials and Methods

*Patients.*

DNA samples were obtained from 512 Japanese chronic hepatitis C patients, after informed consent, recruited from NHO Nagasaki Medical Center, Nagoya City University Hospital, Nagoya Daini Red Cross Hospital, and Kawasaki Medical University Hospital in Japan. Of them, 90 patient's data were also used in the previous paper (15). All of the subjects had undergone a standard course of PEG-IFN-α/RBV therapy and 316 patients had their virological response status established before this study, of which 272 patients had HCV genotype 1. The NVR, transient virological response (TVR) and SVR were defined 24 weeks after PEG-IFN-α/RBV therapy as previously described (8). This study classified the response outcome in two categories; responders (including those with SVR and TVR) and non-responders (including patients with NVR).

Informed consent was obtained from each patient and the study protocol conformed to the ethics guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committee.

*Samples for assay validation.*

To assess intra-assay and inter-assay precision and both coefficients of variation (CV%), 3 different genotype samples (major, minor, and hetero), 2 positive (major and minor) and 1 negative control were run in triplicate during 3 runs (3 different days).The limit of detection was evaluated by analyzing 3 different genotype samples using 2 different DNA concentrations (1ng, and 0.3ng per assay) with the same 3 controls.

*Invader Plus assay.*

The Invader Plus assay, which combines PCR and the Invader reaction, was performed using the LC480II (Roche Diagnostics, Basel, Switzerland). The enzymes used in Invader Plus are native Taq polymerase (Promega, Fitchburg, WI) and cleavase enzyme (Third Wave Technologies, Madison, WI). Primers and allele probes newly designed for InvaderPlus genotyping of *IL28B* SNPs are shown in Table 1 (Third Wave Japan, Tokyo, Japan). The reaction is configured to use PCR primers with a melting temperature (Tm) of 72°C and an Invader detection probe with a target-specific Tm of 63°C. The invader oligonucleotide overlaps the probe by one nucleotide, forming at 63°C an overlap flap substrate for the cleavase enzyme. The first step in Invader Plus is PCR target amplification, in which the reaction is subjected to 18 cycles of a denaturation step (95°C for 15sec) and hybridization and extension steps (70°C for 1 min). At the end of PCR cycling, the reaction mixture is incubated at 99°C for 10min to inactivate the Taq polymerase. Next, the reaction temperature is lowered to 63°C for 15min to permit hybridization of the probe oligonucleotide and formation of the overlap flap structure (Fig. 1). Data were analyzed by endpoint genotyping software (Roche Diagnostics).

*TaqMan PCR assay.*

The rs8099917 polymorphism was determined using TaqMan Pre-Designed SNP Genotyping Assays, as recommended by the manufacturer (Life Technologies, Carlsbad, CA). The Custom TaqMan SNP Genotyping Assay Service MGB probe was used to determine the genotype of rs12979860 (Life Technologies). Each genome DNA sample (10ng) was amplified using the master mix reagent of LightCycler480 Probe Master (Roche Diagnostics). The assays were carried out using the LC480II under the following conditions: 2min at 50°C, 10min at 95°C, 40 cycles: 15 sec at 95°C, and 1

min at 60°C. Data were analyzed by endpoint genotyping software (Roche Diagnostics).

*Direct Sequencing assay.*

Before proceeding to *IL28B* SNP genotyping by the two methods, 105 DNA samples were genotyped using direct sequencing. To determine the SNP genotype of rs8099917 and rs12979860, the specific primer sets, rs8099917F (5'-AAGTAACACTTGTTCCTTRTAAAAGATTCC-3') and rs8099917R (5'-CGCTATAATTAAAGATGTGGGAGAATGCAA-3'), rs12979860F (5'-CACGGTCGTGCCTGTCGTGT-3') and rs12979860R (5'-TGTGCTGTGCCTTCACGCTCCGAGCA-3') were used, respectively (Life Technologies). The amplification products were sequenced directly in both forward and reverse directions with Prism Big Dye (Life Technologies) on an ABI3100 DNA automated sequencer (Life Technologies).

## Results

*Assay Validation*

In this study, the performance characteristics of the assay were determined before experimental testing. To assess the accuracy of *IL28B* SNP genotyping based on both rs8099917 and rs12979860, the results from the Invader Plus genotyping assay were compared with the results from direct sequencing. Of the 105 DNA samples, 70 samples had the major homozygous allele, 30 samples the minor heterozygous and 5 samples the minor homozygous allele for both SNPs by direct sequencing. This gave 100% concordant results for rs12979860 between the 2 assays, although one sample showed different results for rs8099917 with direct sequencing. As a result of direct sequencing, this sample had the major homozygous allele, but the results of the Invader assay and TaqMan assay both showed the minor heterozygous allele. Precision was