In patients with very low CD4 counts (well below the normal range of 800–1200/μL), ART may cause exacerbation of hepatitis due to recovery of cellular immunity, in a phenomenon known as Immune Reconstitution Inflammatory Syndrome (IRIS). In the majority of cases, IRIS is observed within 16 weeks of starting ART. It can be difficult to distinguish between IRIS and drug-induced liver injury.

An issue with ART is the potential for drug-induced liver injury associated with the use of anti-HIV agents, particularly protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitors (NNRTI). The risk of liver injury generally decreases during ongoing ART;[362] it is however more likely in patients with advanced liver fibrosis, and particularly cirrhosis. Cessation of ART or a change in the agents used should be considered if liver injury is detected or hepatic function deteriorates.

Prolonged administration of tenofovir and/or adefovir can lead to renal damage.[363] In the case of tenofovir, this may be irreversible.[364] For this reason, changes in the drug regimen should be considered before the estimated glomerular filtration rate (eGFR) falls below 60% or phosphorus reabsorption falls below 70%.

### 6.4.3 Problems with treatment and responses

Before commencing ART including anti-HBV agents, it is important to check for a history of treatment with anti-HBV agents such as lamivudine, adefovir, entecavir or any of the anti-HIV drugs listed in Table 16. If any of these agents have been administered in the past, an infectious diseases specialist should be consulted regarding the choice of ART agents.

Functional hepatic reserve should also be evaluated prior to commencing ART including anti-HBV agents, given that IRIS can potentially exacerbate hepatitis in patients with a low hepatic reserve. Protease inhibitors and NNRTIs known to cause hepatic dysfunction should be avoided with these patients.

Entecavir is not recommended for patients coinfected with HIV and HBV not being administered anti-HIV agents, as it can lead to the emergence of drug-resistant HIV.

All the abovementioned factors should be considered in selecting the ART regimen. The ART regimen should consist of a backbone of either tenofovir (TDF) with emtricitabine (FTC), or tenofovir (TDF) with lamivudine (3TC), together with a key drug (integrase inhibitor, NNRTI or PI).

Where IRIS occurs during ART including anti-HBV agents, it is usually only transient in nature. Although it is generally held that cessation of ART should be considered when transaminase levels reach more than five to ten times the baseline level, it is preferable to address the problem without interrupting ART.

If it proves necessary to cease administration of an anti-HIV drug with anti-HBV activity (such as lamivudine, emtricitabine, tenofovir or Truvada (emtricitabine+tenofovir)) due to adverse reactions associated with ART, there is a danger of recurrence or aggravation of hepatitis. Where possible, two anti-HBV agents should be administered instead. Consideration should be given to entecavir+adefovir combination therapy.

It is rare for treatment to be indicated for HBV alone, and "treatment of HIV infection not indicated or not wanted". If this situation does arise, Peg-IFNα-2a therapy should be considered.

Specific directions regarding coinfections with HBV and HIV are set out in the HIV Guidelines.[365,366]

*Recommendations*

- *In patients with very low CD4 counts (well below the normal range of 800–1200/μL), ART may exacerbate hepatitis due to recovery of cellular immunity.*
- *When administering ART, we should take into consideration the potential for anti-HIV agents to cause drug-induced liver injury.*
- *Before commencing ART involving anti-HBV agents, it is important to check for a history of treatment with anti-HBV agents.*
- *Before commencing ART involving anti-HBV agents, it is important to evaluate functional hepatic reserve.*
- *The ART regimen should consist of a backbone of either tenofovir (TDF) with emtricitabine (FTC), or tenofovir (TDF) with lamivudine (3TC), together with a key drug (integrase inhibitor, non-nucleoside reverse transcriptase inhibitor or protease inhibitor).*
- *If it is necessary to cease administration of an anti-HIV drug with anti-HBV activity due to adverse reactions associated with ART, there is a danger of recurrence or aggravation of hepatitis. Where possible, two anti-HBV agents should be administered instead. Consideration should be given to entecavir+adefovir combination therapy.*

## CONFLICTS OF INTEREST

THE MEMBERS OF Drafting Committee for Hepatitis Management Guidelines have received consultant

fees from GlaxoSmithKline, royalty from SRL, lecture fees from Ajinomoto Pharmaceuticals, MSD, Daiichi-Sankyo, Dainippon-Sumitomo Pharma, Mitsubishi Tanabe Pharma, Chugai Pharmaceutical, Bristol-Myers-Squibb, and research support from Eisai, MSD, Kan Research Institute, GlaxoSmithKline, Chugai Pharmaceutical, Bristol-Myers-Squibb, Daiichi-Sankyo, Mitsubishi Tanabe Pharma, Dainippon-Sumitomo Pharma, Toray, Minophagen Pharmaceutical.

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# Association of Gene Expression Involving Innate Immunity and Genetic Variation in Interleukin 28B With Antiviral Response

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**Innate immunity plays an important role in host antiviral response to hepatitis C viral (HCV) infection. Recently, single nucleotide polymorphisms (SNPs) of *IL28B* and host response to peginterferon α (PEG-IFNα) and ribavirin (RBV) were shown to be strongly associated. We aimed to determine the gene expression involving innate immunity in *IL28B* genotypes and elucidate its relation to response to antiviral treatment. We genotyped *IL28B* SNPs (rs8099917 and rs12979860) in 88 chronic hepatitis C patients treated with PEG-IFNα-2b/RBV and quantified expressions of viral sensors (*RIG-I, MDA5*, and *LGP2*), adaptor molecule (*IPS-1*), related ubiquitin E3-ligase (*RNF125*), modulators (*ISG15* and *USP18*), and *IL28* (*IFNλ*). Both *IL28B* SNPs were 100% identical; 54 patients possessed rs8099917 TT/rs12979860 CC (*IL28B* major patients) and 34 possessed rs8099917 TG/rs12979860 CT (*IL28B* minor patients). Hepatic expressions of viral sensors and modulators in *IL28B* minor patients were significantly up-regulated compared with that in *IL28B* major patients (≈3.3-fold, $P < 0.001$). However, expression of *IPS-1* was significantly lower in *IL28B* minor patients (1.2-fold, $P = 0.028$). Expressions of viral sensors and modulators were significantly higher in nonvirological responders (NVR) than that in others despite stratification by *IL28B* genotype (≈2.6-fold, $P < 0.001$). Multivariate and ROC analyses indicated that higher *RIG-I* and *ISG15* expressions and *RIG-I/IPS-1* expression ratio were independent factors for NVR. IPS-1 down-regulation in *IL28B* minor patients was confirmed by western blotting, and the extent of IPS-1 protein cleavage was associated with the variable treatment response. *Conclusion:* Gene expression involving innate immunity is strongly associated with *IL28B* genotype and response to PEG-IFNα/RBV. Both *IL28B* minor allele and higher *RIG-I* and *ISG15* expressions and *RIG-I/IPS-1* ratio are independent factors for NVR.** (HEPATOLOGY 2012;55:20-29)

Infection with hepatitis C virus (HCV) is a common cause of chronic hepatitis, which progresses to liver cirrhosis and hepatocellular carcinoma in many patients.[1] Pegylated interferon α (PEG-IFNα) and ribavirin (RBV) combination therapy has been used to treat chronic hepatitis C (CH-C) to alter the natural course of this disease. However, 20% patients are nonvirological responders (NVR) whose HCV-RNA does not become negative during the 48 weeks of PEG-IFNα/RBV combination therapy.[2] In a recent genome-wide association study, single nucleotide polymorphisms (SNPs) located near interleukin 28B

*Abbreviations: CH-C, chronic hepatitis C; γ-GTP, γ-glutamyl transpeptidase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HCV, hepatitis C virus; HMBS, hydroxymethylbilane synthase; IL28, interleukin 28; IPS-1, IFNβ promoter stimulator 1; ISG15, interferon-stimulated gene 15; MDA5, melanoma differentiation associated gene 5; NVR; nonvirological responders; PEG-IFNα, pegylated interferonα; SNP, single nucleotide polymorphism; RIG-I, retinoic acid-inducible gene I; RBV, ribavirin; RNF125, ring-finger protein 125; ROC, receiver operator characteristic; SVR, sustained viral responder; TVR, transient virological responder; USP18, ubiquitin-specific protease 18; VR, virological responder.*

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*Received May 14, 2011; accepted August 16, 2011.*

*Supported by grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology and the Japanese Ministry of Welfare, Health and Labor. The funding source had no role in the collection, analysis, or interpretation of the data, or in the decision to submit the article for publication.*

(*IL28B*) that encodes for type III IFN$\lambda$3 were shown to be strongly associated with a virological response to PEG-IFN$\alpha$/RBV combination therapy.[3-5] In particular, the rs8099917 TG and GG genotypes were shown to be strongly associated with a null virological response to PEG-IFN$\alpha$/RBV.[3] However, mechanisms involving resistance to PEG-IFN$\alpha$/RBV have not been completely elucidated.

The innate immune system has an essential role in host antiviral defense against HCV infection.[6] The retinoic acid-inducible gene I (RIG-I), a cytoplasmic RNA helicase, and related melanoma differentiation associated gene 5 (MDA5) play essential roles in initiating the host antiviral response by detecting intracellular viral RNA.[7,8] The IFN$\beta$ promoter stimulator 1 (IPS-1)—also called the caspase-recruiting domain adaptor inducing IFN$\beta$, mitochondrial antiviral signaling protein, or virus-induced signaling adaptor—is an adaptor molecule. IPS-1 connects RIG-I sensing to downstream signaling, resulting in *IFN$\beta$* gene activation.[9-12] RIG-I sensing of incoming viral RNA has been shown to be modified by LGP2,[8,13] a helicase related to RIG-I and MDA5 lacking caspase-recruiting domain. The ubiquitin ligase ring-finger protein 125 (RNF125) has been shown to conjugate ubiquitin to RIG-I, MDA5, and IPS-1 and this suppresses the functions of these proteins.[14] Further, these molecules are ISGylated by the IFN-stimulated gene 15 (ISG15), a ubiquitin-like protein,[15] and ISG15 is specifically removed from ISGylated protein by ubiquitin-specific protease 18 (USP18) to regulate the RIG-I/IPS-1 system.[16,17] Moreover, the NS3/4A protease of HCV specifically cleaves IPS-1 as part of its immune-evasion strategy.[9,18] Therefore, the RIG-I/IPS-1 system and its regulatory systems have essential roles in the innate antiviral response.

Recently, we demonstrated that baseline intrahepatic gene expression levels of the RIG-I/IPS-1 system were prognostic biomarkers of the final virological outcome in CH-C patients who were treated with PEG-IFN$\alpha$/RBV combination therapy.[19] We found that up-regulation of *RIG-I* and *ISG15* and a higher expression ratio of *RIG-I/IPS-1* could predict NVR for subsequent treatment with PEG-IFN$\alpha$/RBV combination therapy.[19] However, association of gene expression involving innate immunity and genetic variation of *IL28B* has not yet been elucidated. Hence, the aim of this study was to determine gene expression involving the innate immune system in different genetic variations of *IL28B* and elucidate the relation of gene expression to final virological outcome of PEG-IFN$\alpha$/RBV combination therapy in CH-C patients.

## Patients and Methods

***Patients.*** Among histologically proven CH-C patients admitted at the Musashino Red Cross Hospital, 88 patients with HCV genotype 1b and a high viral load (>5 log IU/mL by TaqMan HCV assay; Roche Molecular Diagnostics, Tokyo, Japan) were included in the present study (Table 1). Patients with decompensated liver cirrhosis, autoimmune hepatitis, or alcoholic liver injury were excluded. No patient had tested positive for hepatitis B surface antigen or anti-human immunodeficiency virus antibody or had received immunomodulatory therapy before enrollment. Forty-two patients had been enrolled in a previous study that determined hepatic gene expression involving innate immunity.[19] Written informed consent was obtained from all patients and the study was approved by the Ethical Committee of Musashino Red Cross Hospital in accordance with the Declaration of Helsinki.

***Treatment Protocol.*** The patients were administered subcutaneous injections of PEG-IFN$\alpha$-2b (PegIntron, MSD, Whitehouse Station, NJ) at a dose of 1.5 $\mu$g kg$^{-1}$ week$^{-1}$ for 48 weeks. RBV (Rebetol, MSD) was administered concomitantly over this treatment period, administered orally twice daily at 600 mg/day for patients who weighed less than 60 kg and 800 mg/day for patients who weighed between 60-80 kg. The dose of PEG-IFN$\alpha$-2b was reduced to 0.75 $\mu$g kg$^{-1}$ week$^{-1}$ when either neutrophil count was less than 750/mm$^3$ or platelet count was less than 80 $\times$ 10$^3$/mm$^3$. The dose of RBV was reduced to 600 mg/day when the hemoglobin concentration decreased to 10 g/dL. More than 80% adherence was achieved in all patients.

***Measurement of Hepatic Gene Expression.*** Liver biopsy was performed immediately before initiating

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*DOI 10.1002/hep.24623*

*Potential conflict of interest: Nothing to report.*

*Additional Supporting Information may be found in the online version of this article.*

**Table 1. Patient Characteristics and *IL28B* Genotype**

| | *IL28B* Major* | *IL28B* Minor† | *P*-value‡ |
|---|---|---|---|
| Patients, n | 54 | 34 | |
| Age (SD), year | 58.8 (10.0) | 59.1 (10.3) | 0.918§ |
| Sex, n (%) | | | 0.051‖ |
| Male | 13 (24.1) | 15 (44.1) | |
| Female | 41 (75.9) | 19 (55.9) | |
| BMI (SD), kg/m$^2$ | 22.7 (3.5) | 23.5 (3.6) | 0.193§ |
| ALT (SD), IU/L | 61.3 (50.7) | 62.4 (44.7) | 0.962§ |
| γ-GTP (SD), IU/L | 36.7 (25.9) | 57.3 (52.4) | 0.010§ |
| LDL-cholesterol (SD), mg/dL | 103.3 (29.8) | 91.8 (26.9) | 0.067§ |
| Hemoglobin (SD), g/dL | 14.1 (1.4) | 14.4 (1.3) | 0.186§ |
| Platelet count (SD), $\times 10^3$3/μL | 161 (6.4) | 163 (4.4) | 0.489§ |
| Fibrosis stage, n (%) | | | 0.532‖ |
| F1, 2 | 38 (70.4) | 26 (76.5) | |
| F3, 4 | 16 (29.6) | 8 (23.5) | |
| Viral load (SD), $\times 10^{63}$ IU/mL | 1.7 (1.4) | 1.9 (2.0) | 0.788§ |
| %HCV core 70 & 91 a.a. double mutation¶ | 8.9 | 43.5 | 0.001‖ |
| %ISDR wild** | 43.5 | 51.7 | 0.486‖ |
| Viral response, n (%) | | | <0.001‖ |
| SVR | 17 (31.5) | 13 (38.2) | |
| TVR | 26 (48.1) | 3 (8.8) | |
| NVR | 11 (20.4) | 18 (52.9) | |

Unless otherwise indicated, data are given as mean (SD).

*rs8099917 TT and rs12979860 CC.

†rs8099917 TG and rs12979860 CT.

BMI, body mass index; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; LDL-C, low-density lipoprotein cholesterol; HCV, hepatitis C virus; ISDR, interferon sensitivity determining region; SVR, sustained virological response; TVR, transient virological response; NVR, nonvirological response.

‡Comparison between *IL28B* major and minor genotypes.

§Mann-Whitney *U* test.

‖Chi-square test.

¶HCV core mutation was determined in 68 patients.

**ISDR was determined in 75 patients.

the therapy. After extraction of total RNA from liver biopsy specimens, the messenger RNA (mRNA) expression of the positive and negative cytoplasmic viral sensor (*RIG-I, MDA5*, and *LGP2*), the adaptor molecule (*IPS-1*), the related ubiquitin E3-ligase (*RNF125*), the modulators of these molecules (*ISG15* and *USP18*), and *IFNλ* (*IL28A/B*) was quantified by real-time quantitative polymerase chain reaction (PCR) using target gene-specific primers. In brief, total RNA was extracted by the acid-guanidinium-phenol-chloroform method using Isogen reagent (Nippon Gene, Toyama, Japan) from the liver biopsy specimen, which was 0.2-0.4 cm in length and 13G in diameter. Complementary DNA (cDNA) was transcribed from 2 μg of total RNA template in a 140-μL reaction mixture using the SYBR RT-PCR Kit (Takara Bio, Otsu, Japan) with random hexamer. Real-time quantitative PCR was performed using Smart Cycler version II (Takara Bio) with the SYBR RT-PCR Kit (Takara Bio) according to the manufacturer's instructions. Assays were performed in duplicate and the expression levels of target genes were normalized to the expressions of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene and hydroxymethylbilane synthase (*HMBS*), an enzyme that is stable in the liver, as quantified using real-time quantitative PCR as internal controls. For accurate normalization, a set of two housekeeping genes was used in the present study. Sequences of the primer sets were as follows: *RIG-I*, 5′-AAAGCATGCA TGGTGTTCCAGA-3′, 5′-TCATTCGTGCATGCTC ACTGATAA-3′; *MDA5*, 5′-ACATAACAGCAACATG GGCAGTG-3′, 5′-TTTGGTAAGGCCTGAGCTGG AG-3′; *LGP2*, 5′-ACAGCCTTGCAAACAGTACAAC CTC-3′, 5′-GTCCCAAATTTCCGGCTCAAC-3′; *IPS-1*, 5′-GGTGCCATCCAAAGTGCCTACTA-3′, 5′-CAGC ACGCCAGGCTTACTCA-3′; *RNF125*, 5′-AGGGCA CATATTCGGACTTGTCA-3′, 5′-CGGGTATTAAAC GGCAAAGTGG-3′; *ISG15*, 5′-AGCGAACTCATCT TTGCCAGTACA-3′, 5′-CAGCTCTGACACCGACA TGGA-3′; *USP18*, 5′-TGGTTCTGCTTCAATGACT CCAATA-3′, 5′-TTTGGGCATTTCCATTAGCACT C-3′; *IFNλ*: 5′-CAGCTGCAGGTGAGGGA-3′, 5′-G GTGGCCTCCAGAACCTT-3′; *GAPDH*, 5′-GCACC GTCAAGGCTGAGAAC-3′, 5′-ATGGTGGTGAAGA CGCCAGT-3′; *HMBS*, 5′-AAGCGGAGCCATGTCT GGTAAC-3′, 5′-GTACCCACGCGAATCACTCTCA-3′.

***Genotyping for* IL28B *(rs8099917 and rs12979860) Polymorphism.*** Genetic polymorphism in a tagged SNP located near the *IL28B* gene (rs8099917 and rs12979860) was determined by direct sequencing of PCR-amplified DNA. In brief, after extraction from whole blood samples, genomic DNA was amplified by PCR. Sequences of the primer sets were: rs8099917, 5′-ATCCTCCTCTCATCCCTCA TC-3′, 5′-GGTATCAACCCCACCTCAAAT-3′; rs129 79860, 5′-GGACGAGAGGGCGTTAGAG-3′, 5′-AG GGACCGCTACGTAAGTCAC-3′.

Both strands of the PCR products were sequenced by the dye terminator method using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Chiba, Japan); nucleotide sequences were determined by a capillary DNA sequencer ABI3730xl (Applied Biosystems). Homozygosity (rs8099917 GG and rs12979860 TT) or heterozygosity (rs8099917 TG and rs12979860 CT) of the minor sequence was defined as having the *IL28B* minor allele, whereas homozygosity for the major sequence (rs8099917 TT and rs12979860 CC) was defined as having the *IL28B* major allele.

***Western Blotting.*** Western blotting was performed using samples from 14 patients (six from *IL28B* major patients and eight from *IL28B* minor patients) as described.[19] In brief, liver biopsy specimens of

approximately 10 mg were homogenized in 100 μL of Complete Lysis-M (Roche Applied Science, Penzberg, Germany). Next, 30 μg of protein was separated by NuPAGE 4%-12% Bis-Tris gels (Invitrogen, Carlsbad, CA) and blotted on polyvinylidene difluoride membranes. The membranes were immunoblotted with anti-RIG-I (Cell Signaling Technology, Danvers, MA) or anti-IPS-1 (Enzo Life Science, Farmingdale, NY), followed by anti-β-actin (Sigma Aldrich, St. Louis, MO). After immunoblotting with horseradish peroxidase-conjugated secondary antibody, signals were detected by chemiluminescence (BM Chemiluminescence Blotting Substrate, Roche Applied Science, Mannheim, Germany). Optical densitometry was performed using ImageJ software (NIH, Bethesda, MD). Naive Huh7 cells were used for a positive control for full-length IPS-1, and cells transfected with HCV-1b subgenomic replicon[20] were used for a positive control for cleaved IPS-1.

***Definitions of Response to Therapy.*** A patient negative for serum HCV-RNA during the first 6 months after completing PEG-IFNα-2b/RBV combination therapy was defined as a sustained viral responder (SVR), and a patient for whom HCV-RNA became negative at the end of therapy and reappeared after completion of therapy was defined as a transient virological responder (TVR). A patient for whom HCV-RNA became negative at the end of therapy (SVR + TVR) was defined as a virological responder (VR). A patient whose HCV-RNA did not become negative during the course of therapy was defined as an NVR. HCV-RNA was determined by TaqMan HCV assay (Roche Molecular Diagnostics).

***Statistical Analysis.*** Categorical data were compared using the chi-square test and Fisher's exact test. Distributions of continuous variables were analyzed by the Mann-Whitney *U* test for two groups. All tests of significance were two-tailed and $P < 0.05$ was considered statistically significant.

## Results

***Patient Characteristics and* IL28B *Genotype.*** Table 1 shows patient characteristics according to *IL28B* genotype. SNPs at rs8099917 and rs12979860 were 100% identical; 54 patients were identified as having the major alleles (rs8099917 TT/rs12979860 CC; *IL28B* major patients) and the remaining 34 had the minor alleles (rs8099917 TG/rs12979860 CT; *IL28B* minor patients). Patients having a minor homozygote (rs8099917 GG or rs12979860 TT) were not found in this study, which is consistent with a recent report of the rarity of a minor homozygote in Japanese patients.[3] *IL28B* minor patients were significantly associated with a higher γ-glutamyl transpeptidase (γ-GTP) level and higher frequency of mutations at amino acid positions 70 and 91 of the HCV core region (glutamine or histidine mutation at amino acid position 70; methionine mutation at amino acid position 91). NVR rate was significantly higher in *IL28B* minor patients than in *IL28B* major patients.

***Gene Expression Involving Innate Immunity and* IFNλ *in the Liver.*** Hepatic expression levels of cytoplasmic viral sensors (*RIG-I, MDA5*, and *LGP2*) were significantly higher in *IL28B* minor patients than in *IL28B* major patients (Fig. 1). Similarly, expressions of *ISG15* and *USP18* were significantly higher in *IL28B* minor patients than in *IL28B* major patients (Fig. 1). In contrast, the hepatic expression of the adaptor molecule (*IPS-1*) was significantly lower in *IL28B* minor patients than that in *IL28B* major patients (Fig. 1). Hepatic expression of *RNF125* was similar among *IL28B* genotypes (Fig. 1). *IFNλ (IL28A/B)* expression was higher in *IL28B* minor patients, but not statistically significant (Fig. 1). Because expression of *RIG-I* and *IPS-1* were negatively correlated, the expression ratio of *RIG-I/IPS-1* in *IL28B* minor patients was significantly higher than in *IL28B* major patients (Fig. 1).

Next, to assess the relationship between baseline hepatic gene expression and treatment efficacy, we compared levels of gene expression involving innate immunity and *IFNλ* based on the final virological response (Fig. 2). Overall, hepatic expressions of cytoplasmic viral sensors and the ISG15/USP18 system in NVR patients were significantly higher than those in VR patients. In a similar but opposite manner, hepatic expressions of *IPS-1* and *RNF125* in NVR patients were significantly lower than that in VR patients, and the expression of *IFNδ* was higher in NVR patients, but the differences were not statistically significant. Expression ratio of *RIG-I/IPS-1* was significantly higher in NVR patients than that in VR patients.

Because hepatic expressions of the RIG-I/IPS-1 and ISG15/USP18 systems were significantly related both to *IL28B* minor and NVR patients, *RIG-I* and *ISG15* expression levels and the *RIG-I/IPS-1* ratio between VR and NVR patients were further stratified by *IL28B* genotype (Fig. 3). Even in the subgroup of *IL28B* minor patients, the expressions of *RIG-I* and *ISG15* were significantly higher in NVR patients than those in VR patients. Similar tendencies were observed in a subgroup of *IL28B* major patients, in whom the *RIG-I/IPS-1* expression ratio was significantly higher in

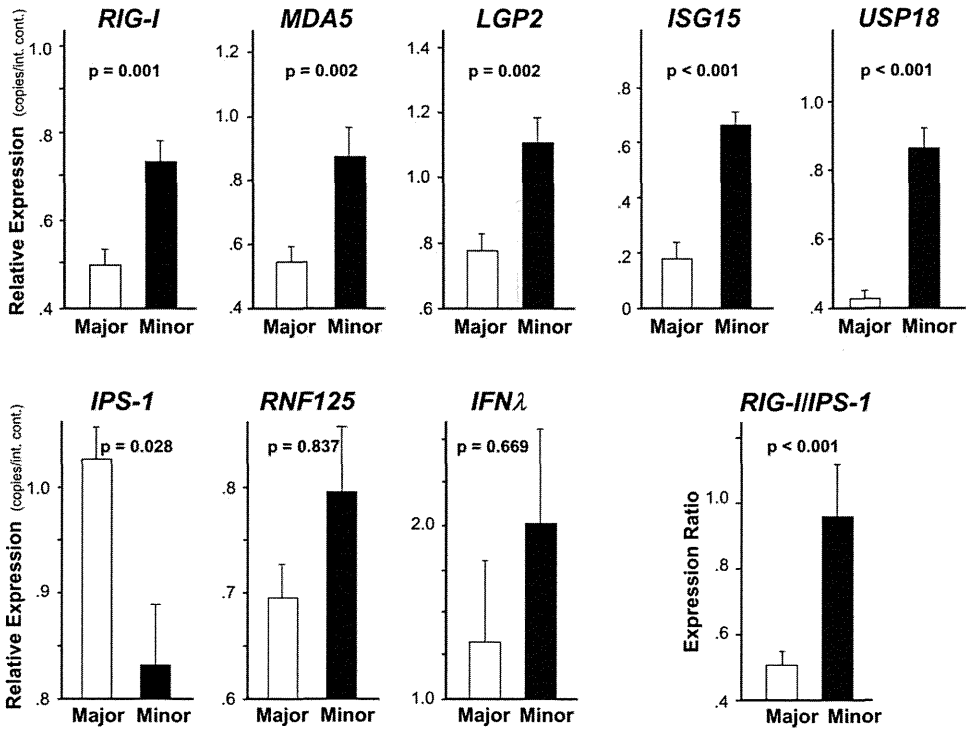


Fig. 1. Comparison of hepatic gene expression levels between *IL28B* major (rs8099917 TT/rs12979860 CC, n = 54) and *IL28B* minor patients (rs8099917 TG/rs12979860 CT, n = 34). Expression levels of cytoplasmic viral sensors (*RIG-I*, *MDA5*, and *LGP2*), modulators (*ISG15* and *USP18*), an adaptor (*IPS-1*), negative regulators (*RNF125*) and *IFNλ*, and expression ratio of the *RIG-I/IPS-1* are shown. Error bars indicate standard error. The *P*-values were determined by the Mann-Whitney *U* test.

NVR patients than in VR patients. However, in patients of the same virological response subgroup, *RIG-I* and *ISG15* expression levels and *RIG-I/IPS-1* ratio were higher in *IL28B* minor patients, and the difference in *ISG15* expression in subgroup of VR and NVR patients and that in *RIG-I/IPS-1* ratio in subgroup of VR patients was statistically significant between *IL28B* genotypes (Fig. 3).

***Receiver Operator Characteristic (ROC) Analysis.*** To determine the usefulness of these gene quantifications and *IL28B* genotyping as predictors of NVR, an ROC analysis was conducted (Fig. 4A). The area under the ROC curve for *RIG-I* and *ISG15* expressions and *RIG-I/IPS-1* expression ratio was 0.712, 0.782, and 0.732, respectively, suggesting that quantification of these gene transcripts is useful for

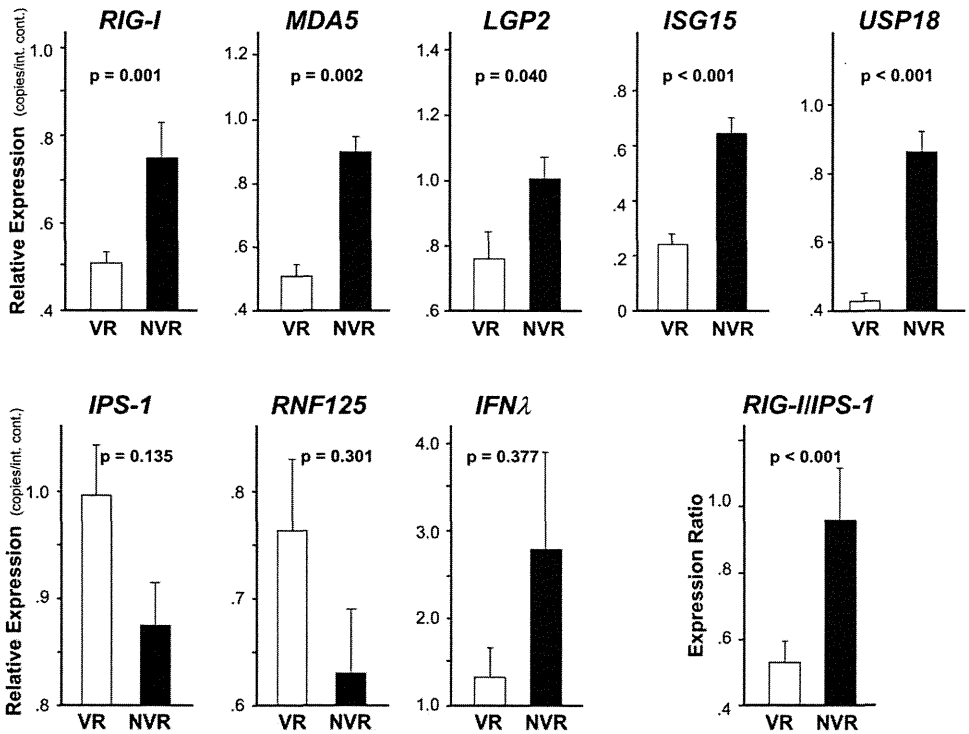


Fig. 2. Comparison of hepatic gene expression levels between virological responders (VR, n = 60) and nonvirological responders (NVR, n = 28). Expression levels of cytoplasmic viral sensors (*RIG-I*, *MDA5*, and *LGP2*), modulators (*ISG15* and *USP18*), an adaptor (*IPS-1*), negative regulators (*RNF125*) and *IFNλ*, and *RIG-I/IPS-1* expression ratio are shown. Error bars indicate standard error. The *P*-values were determined by the Mann-Whitney *U* test.

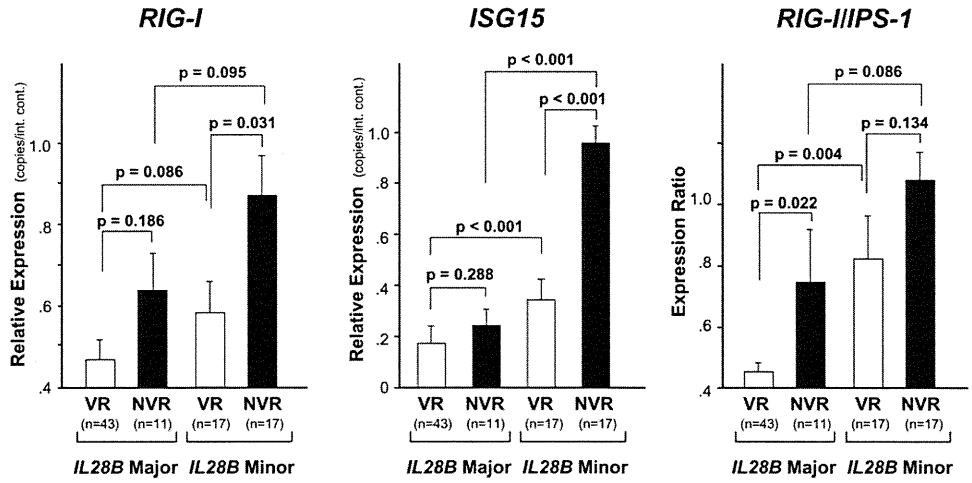


Fig. 3. Comparison of hepatic gene expression levels between virological responders (VR) and nonvirological responders (NVR) in subgroups of the *IL28B* genotype (*IL28B* Major, rs8099917 TT/rs12979860 CC; *IL28B* Minor, rs8099917 TG/rs12979860 CT). Expressions of *RIG-I* and *ISG15* as well as the *RIG-I/IPS-1* expression ratio are shown. Error bars indicate standard error. The numbers of patients in each subgroup are shown in the bottom of the figure.

prediction of NVR (Table 2). The area under the ROC curve for *IL28B* genotype was 0.662, which was lower compared with that for *RIG-I* and *ISG15* expressions and *RIG-I/IPS-1* ratio.

When we stratified the patients by the cutoff value for *RIG-I* and *ISG15* expressions and *RIGI/IPS-1* ratio, no statistically significant difference was found in NVR rates among *IL28B* genotypes within the same subgroup (Fig. 4B).

***Factors Associated with NVR.*** In univariate analysis, age, platelet counts, double mutation at amino acid positions 70 and 91 of the HCV core region, *IL28B* minor allele, and hepatic expressions of *RIG-I, MDA5, LGP2, ISG15*, and *USP18*, and *RIG-I/IPS-1* ratio were significantly

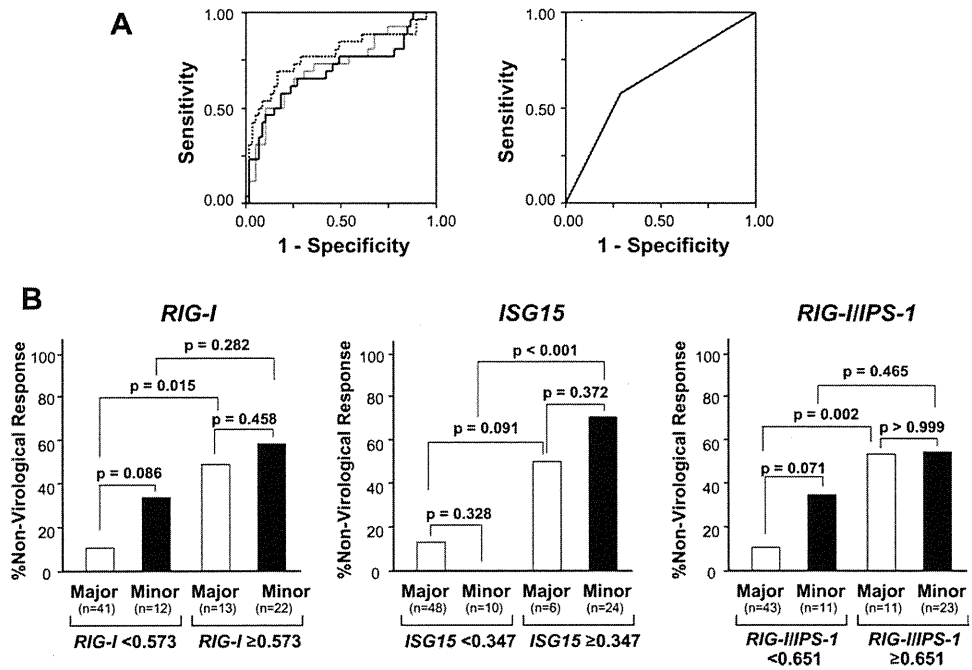


Fig. 4. (A) Receiver operator characteristics (ROC) curve for prediction of nonvirological response. ROC curves were generated to compare *RIG-I* (black line), *ISG15* (dotted line), and *RIG-I/IPS-1* ratio (gray line) (all in the left panel), and *IL28B* genotype (in the right panel). (B) Nonvirological response rate in *IL28B* major (rs8099917 TT/rs12979860 CC) and minor patients (rs8099917 TG/rs12979860 CT) in subgroups divided by the cutoff value of *RIG-I* and *ISG15* expression and the *RIG-I/ISG15* ratio determined by ROC analysis. Cutoff values of *RIG-I* and *ISG15* expression are expressed as expression copy number normalized to the expression of an internal control. The numbers of patients in each subgroup are shown in the bottom of the figure.