**KIR, HLA, and IL28B Variant Predict Response to Antiviral Therapy in Genotype 1 Chronic Hepatitis C Patients in Japan**

Yuichi Nozawa\(^1\)*, Takeji Umemura\(^1\)*, Satoru Joshita\(^1\), Yoshihiko Katsuyama\(^2\), Soichiro Shibata\(^1\), Takefumi Kimura\(^1\), Susumu Morita\(^1\), Michiharu Komatsu\(^1\), Akihiro Matsumoto\(^1\), Eiji Tanaka\(^1\), Masao Ota\(^3\)

1 Division of Hepatology and Gastroenterology, Department of Medicine, Shinshu University School of Medicine, Matsumoto, Japan, 2 Department of Pharmacy, Shinshu University Hospital, Matsumoto, Japan, 3 Department of Legal Medicine, Shinshu University School of Medicine, Matsumoto, Japan

### Abstract

Natural killer cell responses play a crucial role in virus clearance by the innate immune system. Although the killer immunoglobulin-like receptor (KIR) in combination with its cognate human leukocyte antigen (HLA) ligand, especially KIR2DL3-HLA-C1, is associated with both treatment-induced and spontaneous clearance of hepatitis C virus (HCV) infection in Caucasians, these innate immunity genes have not been fully clarified in Japanese patients. We therefore investigated 16 KIR genotypes along with HLA-B and -C ligands and a genetic variant of interleukin (IL) 28B (rs8099917) in 115 chronic hepatitis C genotype 1 patients who underwent pegylated-interferon-α2b (PEG-IFN) and ribavirin therapy. HLA-Bw4 was significantly associated with a sustained virological response (SVR) to treatment (P = 0.017; odds ratio [OR] = 2.50, ), as was the centromeric A/A haplotype of KIR (P = 0.015; OR = 3.37). In contrast, SVR rates were significantly decreased in patients with KIR2DL2 or KIR2DS2 (P = 0.015; OR = 0.30, and P = 0.025; OR = 0.32, respectively). Multivariate logistic regression analysis subsequently identified the IL28B TT genotype (P = 0.00009; OR = 6.87, 95% confidence interval [CI] = 2.62 - 18.01), KIR2DL2/HLA-C1 (P = 0.014; OR = 0.24, 95% CI = 0.08 - 0.75), KIR3DL1/HLA-Bw4 (P = 0.008, OR = 3.32, 95% CI = 1.37 - 8.05), and white blood cell count at baseline (P = 0.009; OR = 3.32, 95% CI = 1.35 - 8.16) as independent predictive factors of an SVR. We observed a significant association between the combination of IL28B TT genotype and KIR3DL1-HLA-Bw4 in responders (P = 0.0019), whereas IL28B TT along with KIR2DL2/HLA-C1 was related to a non-response (P = 0.0067). In conclusion, combinations of KIR3DL1/HLA-Bw4, KIR2DL2/HLA-C1, and a genetic variant of the IL28B gene are predictive of the response to PEG-IFN and ribavirin therapy in Japanese patients infected with genotype 1b HCV.

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* E-mail: tumemura@shinshu-u.ac.jp (TU); otamasao@shinshu-u.ac.jp (MO)

☯ These authors contributed equally to this work.

### Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide. Chronic HCV infection often develops into chronic hepatitis, which may progress to liver cirrhosis and/or hepatocellular carcinoma (HCC)[1]. HCC is a leading cause of death from malignant neoplasms in Japan[2]. Since approximately 70% of Japanese HCC patients are infected with HCV, the successful eradication of this virus, defined as a sustained virological response (SVR), is considered important to decrease the incidence of HCC.

Natural killer (NK) cells are key components of the innate antiviral immune response that are controlled by a balance of activation and inhibitory receptors. NK cell activation receptors include C-type lectin-like receptors (NKG2C, NKG2D, and NKG2E), natural cytotoxicity receptors (NKP30, NKP44, and NKP46), and CD16, while known inhibitory receptors include killer cell immunoglobulin-like receptors (KIRs) and the CD94/NKG2 family, which also contains a C-type lectin-like receptor (NKG2A) [3,4]. Sixteen KIR genes and pseudogenes have been identified that are encoded by a family of genes located on human chromosome 19q13.4. One particular feature of KIRs is their substantial genetic diversity. Some inhibitory KIRs
recognize human leukocyte antigen (HLA) class I molecules as their ligands; KIR2DL1 recognizes HLA-C group 2 (HLA-C2) allotypes having lysine at amino acid position 80, whereas KIR2DL2 and KIR2DL3 recognize HLA-C group 1 (HLA-C1) allotypes having asparagine at amino acid position 80 [5]. KIR2DL2 and KIR2DL3 also recognize HLA-B*4601 acquiring the C1 epitope by gene conversion [6]. Furthermore, KIR3DL1 recognizes subsets of HLA-A and HLA-B allotypes having the βw4 epitope determined by amino acid positions 77-83 [7].

It has been well documented that certain KIR–HLA receptor-ligand combinations are associated with susceptibility to infectious diseases, such as HCV, as well as with disease progression and treatment response [8-15]. Recent reports have also identified a relationship between interleukin (IL) 28B gene polymorphisms and treatment and spontaneous resolution of HCV infection [16-19]. Dring et al. observed that the presence of IL28B gene polymorphisms and KIR genotypes synergized to increase the risk of chronic HCV infection [20], although this finding is under debate [21]. Suppiah et al. [22] recently reported that genotyping for IL28B, HLA-C, and KIR genes was useful for predicting HCV treatment response in patients of European descent. As these gene associations have not yet been studied in the Japanese population, we evaluated whether HLA-KIR interactions, in addition to an IL28B polymorphism, would influence the outcome of pegylated-interferon-α (PEG-IFN) and ribavirin therapy in Japanese patients with chronic hepatitis C.

Materials and Methods

Ethics statement

This study was approved by the ethical committee of Shinshu University School of Medicine, Matsumoto, Japan, and written informed consent was obtained from all participants. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Subjects

One hundred and fifteen consecutive IFN-treatment-naïve patients with chronic hepatitis C were enrolled in this study. All subjects were seen at Shinshu University Hospital or one of its affiliated hospitals. The clinical and demographic characteristics of our cohort are shown in Table 1. Diagnosis of chronic hepatitis C was based on previously reported criteria [23]: 1) presence of serum HCV antibodies and detectable viral RNA; 2) absence of detectable hepatitis B surface antigen and antibody to the human immunodeficiency virus; and 3) exclusion of other causes of chronic liver disease or a history of decompensated cirrhosis or HCC. Serum levels of HCV RNA were determined using Cobas Amplicor assays (sensitivity: 50 IU/mL; Roche Diagnostic Systems, Tokyo, Japan). HCV genotypes were determined using INNO-LIPA HCV II kits (Innogenetics, Gent, Belgium). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and other relevant biochemical tests were performed using standard methods[24]. Liver fibrosis was assessed using the AST to platelet ratio index (APRI) in this study. APRI has been recognized as a noninvasive test to estimate the degree of liver fibrosis in chronic liver disease with HCV infection [25]. APRI was calculated for all study subjects as follows: AST/upper limit of normal (45 IU/L) × 100/platelet count (10^12/L). Patients received PEG-IFN-α2b (Peginteron; MSD KK, Tokyo, Japan; 1.5 μg/kg of body weight by subcutaneous injection once per week) and ribavirin (Rebetol; MSD KK; 600-1000 grams daily, according to body weight) for 48 weeks, as described previously [26]. Patients achieving a sustained HCV response were defined as those whose serum HCV RNA was undetectable 24 weeks after completing therapy. Patients who did not meet this criterion, who included non-responders and relapers, were regarded as treatment failures.

Table 1. Clinical features of sustained and non-sustained virological response patients with chronic hepatitis C.

| Characteristic                | All (n = 115) | SVR (n = 56) | Non-SVR (n = 59) | P      |
|------------------------------|--------------|-------------|-----------------|--------|
| Age (yr)                     | 60 (24 - 80) | 59 (25 - 80) | 60 (24 - 75)    | 0.43   |
| Male                         | 66 (57)      | 34 (61)     | 32 (54)         | 0.48   |
| Alanine aminotransferase (IU/L) | 46 (17 - 389) | 48 (17 - 389) | 45 (17 - 309)    | 0.81   |
| Aspartate aminotransferase (IU/L) | 43 (17 - 246) | 42 (17 - 231) | 43 (17 - 246)    | 0.49   |
| White blood cells (μL)       | 4410 (2280 - 8240) | 4740 (2700 - 8170) | 4070 (2280 - 8240) | 0.011  |
| Hemoglobin (g/dL)            | 14.4 (9.2 - 18.2) | 15.1 (11.0 - 18.2) | 13.9 (9.2 - 17.4) | 0.002  |
| Platelet count (10^12/μL)    | 15.9 (6.7 - 33.8) | 16.6 (6.3 - 26.2) | 15.6 (6.7 - 33.6) | 0.30   |
| APRI                         | 0.89 (0.21 - 5.40) | 0.59 (0.22 - 5.40) | 0.66 (0.21 - 5.06) | 0.41   |
| HCV RNA (log_{10} IU/mL)     | 6.4 (5.0 - 7.3) | 6.1 (5.0 - 6.8) | 6.5 (5.0 - 7.3) | < 0.001 |

Data are expressed as median (range) or n (%) as appropriate. SVR, sustained virological response; HCV, hepatitis C virus.

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HKA, KIR, and IL28B (rs8099917) Genotyping

Genomic DNA was isolated from whole blood samples using QuickGene-800 assays (Fujifilm, Tokyo, Japan). We genotyped HLA-B, HLA-C, and KIR using a Luminex multi-analyzer profiling system with a LAB type® HD and KIR SSO genotyping kit (One Lambda, Inc., Canoga Park, CA), which is based on PCR sequence-specific oligonucleotide probes[27]. Subjects were identified as having the B/x or A/A genotype as defined previously [28]. Genotypes for the centromeric (Cen) and telomeric (Tel) parts of the KIR locus were determined according to the presence or absence of one or more B haplotype-defining KIR genes. Thus, Cen-A1 and Tel-A1 were the centromeric and telomeric motifs, respectively, of the canonical A KIR haplotype in the present study, Cen-B1 and Cen-B2 were alternative centromeric motifs of common B KIR haplotypes, and Tel-B1 was the common telomeric motif of B haplotypes[29]. For much of this analysis, Cen-B1 and -B2 were grouped together as Cen-B, whereas Cen-A1 was shortened to Cen-A and Tel-A1 to Tel-A, as reported...
Statistical Analysis

The Mann-Whitney U test was employed to analyze continuous variables. Pearson’s chi-squared test was used for the analysis of categorical data. We adopted Fisher’s exact test when the number of subjects was less than 5. The Bonferroni correction for multiple testing was applied to our data of KIR-HLA combinations using the number of comparisons performed by our primary factors of interest in Table 2 (i.e., 8 tests = 4 combinations × 2 comparisons between two groups). A P value of < 0.05 was considered to be statistically significant. Association strength was estimated by calculating the odds ratio (OR) and 95% confidence interval (CI). Our model was corrected for multiple testing when the number of subjects was less than 5. The Bonferroni test was employed to analyze frequencies, we observed that virologic clearance with HCV viral load at baseline was significantly higher than those in the non-SVR group prior to treatment. HCV viral load at baseline was significantly associated with treatment outcome (P < 0.001).

Association of HLA and KIR with a Sustained Virological Response

We first determined the frequency of HLA-Bw and HLA-C alleles in SVR and non-SVR patients (Figure 1). The frequency of HLA-Bw4/Bw6 in responders was significantly higher than that in non-responders (55% [31/56] vs. 36% [21/59]; P = 0.033; OR = 2.24, 95% CI = 1.06 - 4.75). Conversely, patients with the HLA-Bw6 homozygote had a higher non-SVR rate (32% [18/56] vs. 54% [32/59]; P = 0.017; OR = 0.40, 95% CI = 0.19 - 0.85). Overall, HLA-Bw4 was associated with an SVR among patients (68% [38/56] vs. 46% [27/59]; P = 0.017; OR = 2.50, 95% CI = 1.17 - 5.35). The frequencies of HLA-C were not statistically significant. We further checked whether particular HLA-Bw or HLA-C alleles were beneficial to treatment outcome. The HLA-B*35:01 allele was more frequently found in patients with an SVR than in those without (13% [15/102] vs. 4% [5/118]; P = 0.014 [Pc = 0.36]; OR = 3.49, 95% CI = 1.23 - 9.97).

Table 2. Frequency of IL28B genotype, KIR3DL1/HLA-Bw4, and KIR2DL2/HLA-C1 combinations in 56 patients with a sustained virological response (SVR) and 59 patients with a non-SVR to pegylated interferon and ribavirin therapy of chronic hepatitis C.

| KIR3DL1/HLA-Bw4 | KIR2DL2/HLA-C1 | SVR | Non-SVR | P (Pc) | OR (95% CI) |
|-----------------|----------------|-----|---------|--------|-------------|
| +/+             | C1             | (n = 56) | (n = 59) |        |             |
| +/+             | SVR            | 5 (9%) | 7 (12%) | 0.61   |             |
| +/+             | Non-SVR        | 31 (55%) | 19 (32%) | 0.012 (0.1) | 2.61 (1.22 - 5.58) |
| Other           | +/+            | 1 (2%) | 10 (17%) | 0.014 (0.12) | 0.09 (0.01 - 0.72) |
| Other           | Other          | 19 (34%) | 23 (39%) | 0.57   |             |
| IL28B           | KIR3DL1/HLA-Bw4 | SVR | Non-SVR | P (Pc) | OR (95% CI) |
| (n = 56)        | (n = 59)       |     |         |        |             |
| TT              | +/+            | 27 (48%) | 13 (22%) | 0.003 (0.024) | 3.29 (1.47 - 7.39) |
| TT              | Other          | 17 (30%) | 14 (24%) | 0.42   |             |
| TG/GG           | Other          | 9 (16%) | 13 (22%) | 0.42   |             |
| TG/GG           | Other          | 3 (5%) | 19 (32%) | 0.00062 (0.0005) | 0.12 (0.03 - 0.43) |
| IL28B           | KIR2DL2/HLA-C1 | SVR | Non-SVR | P (Pc) | OR (95% CI) |
| (n = 56)        | (n = 59)       |     |         |        |             |
| TT              | Other          | 38 (68%) | 18 (31%) | 0.00062 (0.0005) | 4.81 (2.19 - 10.58) |
| TT              | +/+            | 6 (11%) | 9 (15%) | 0.47   |             |
| TG/GG           | Other          | 12 (21%) | 24 (41%) | 0.026 (0.21) | 0.40 (0.17 - 0.91) |
| TG/GG           | +/+            | 0 (0%) | 8 (14%) | 0.013 (0.1) | -             |

Data are expressed as n (%).

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Results

Patient Characteristics and Treatment Outcome

All patients in our test cohort were infected with HCV genotype 1b. Of the 115 patients receiving PEG-IFN-α2b and ribavirin therapy, 56 (49%) achieved an SVR. The remaining 59 patients were non-responders, 28 of whom experienced a relapse and 31 who were null responders. The median white blood cell count (P = 0.011) and hemoglobin value (P = 0.002) in the SVR group were significantly higher than those in the non-SVR group prior to treatment. HCV viral load at baseline was significantly associated with treatment outcome (P < 0.001).

Association of HLA and KIR with a Sustained Virological Response

We first determined the frequency of HLA-Bw and HLA-C alleles in SVR and non-SVR patients (Figure 1). The frequency of HLA-Bw4/Bw6 in responders was significantly higher than that in non-responders (55% [31/56] vs. 36% [21/59]; P = 0.033; OR = 2.24, 95% CI = 1.06 - 4.75). Conversely, patients with the HLA-Bw6 homozygote had a higher non-SVR rate (32% [18/56] vs. 54% [32/59]; P = 0.017; OR = 0.40, 95% CI = 0.19 - 0.85). Overall, HLA-Bw4 was associated with an SVR among patients (68% [38/56] vs. 46% [27/59]; P = 0.017; OR = 2.50, 95% CI = 1.17 - 5.35). The frequencies of HLA-C were not statistically significant. We further checked whether
Figure 1. Frequency of HLA-Bw and -C alleles in 56 patients with a sustained virological response (SVR) and 59 patients with a non-SVR to pegylated interferon and ribavirin therapy of chronic hepatitis C.

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Figure 2. Frequency of each KIR gene in 56 patients with a sustained virological response (SVR) and 59 patients with a non-SVR to pegylated interferon and ribavirin therapy of chronic hepatitis C.

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26% [6/23], OR = 0.015; OR = 3.37, 95% CI = 1.22 - 9.33). There were no significant differences regarding AA genotype and Tel.

We next analyzed combinations of activation/inhibitory KIRs and their HLA ligands for possible associations with an SVR. Among the combinations of KIR3DL1-HLA-Bw4, KIR2DL2-HLA-C1, and KIR2DL1-HLA-C2, patients who carried the inhibitory KIR3DL1 receptor and its ligand HLA-Bw4 had a significantly higher response rate than those without KIR3DL1 or HLA-Bw4 (58% [36/62] vs. 38% [20/53]; P = 0.030 [Pc = 0.12]; OR = 2.29, 95% CI = 1.08 - 4.84). In contrast, the KIR2DL2-HLA-C1 combination resulted in a significantly lower SVR rate (26% [6/23] vs. 54% [50/92]; P = 0.015 [Pc = 0.06]; OR = 0.30, 95% CI = 0.11 - 0.82). Although several studies have found that KIR2DL3-HLA-C1 carriers are associated with treatment-induced and spontaneous clearance of HCV in Caucasians, no such association was found in our cohort (data not shown).

Patients with KIR3DL1-HLA-Bw4 but without KIR2DL2-HLA-C1 had a higher SVR rate (55% [31/56] vs. 32% [19/59]; P = 0.012 [Pc = 0.1]; OR = 2.61, 95% CI = 1.22 - 5.58) (Table 2). Conversely, the frequency of the KIR2DL2-HLA-C1 positive, but KIR3DL1-HLA-Bw4 negative condition was significantly higher in non-responders (17% [10/59] vs. 2% [1/56]; P = 0.014 [Pc = 0.12]; OR = 0.09, 95% CI = 0.01 - 0.72).

### Prediction of a Sustained Virological Response by KIR-HLA and IL28B

Examination of the IL28B rs8099917 SNP in our cohort revealed significant differences in SVR frequencies. The SVR rate in patients with the IL28B TT genotype was significantly higher in those with TG or GG genotypes (62% [4/44] vs. 27% [12/44], P = 0.003; OR = 4.35, 95% CI = 1.92 - 9.85). In subjects with IL28B TT and KIR3DL1-HLA-Bw4, virologic clearance was significantly increased over other combinations (68% [27/40] vs. 39% [29/75]; P = 0.003 [Pc = 0.024]; OR 3.29, 95% CI = 1.47 - 7.39).

We next evaluated several factors found in association with an SVR to PEG-IFN and ribavirin therapy for independence by logistic regression analysis. Fifty-six responders were selected. Sixty-six responders were compared with 59 non-responders by means of a forward stepwise likelihood ratio logistic regression method; estimated OR coefficients, 95% CI, and P values are summarized in Table 3 for the variables that remained in equation at the last step. IL28B TT genotype (P = 0.00009; OR = 6.87, 95% CI = 2.62 - 18.01), KIR2DL2-HLA-C1 (P = 0.014; OR = 2.24, 95% CI = 0.80 - 7.54), white blood cell count ≥ 4410μL (P = 0.003; OR = 3.32, 95% CI = 1.35 - 8.16), and KIR3DL1-HLA-Bw4 (P = 0.008; OR = 3.32, 95% CI = 1.37 - 8.05) were all identified as independent parameters that significantly influenced an SVR.

The frequency of the IL28B TT genotype with KIR3DL1-HLA-Bw4 in responders was significantly higher than in non-responders (48% [27/56] vs. 22% [13/59]; P = 0.003 [Pc = 0.024]; OR = 3.29, 95% CI = 1.47 - 7.39) (Table 2). Patients with the IL28B TT genotype without KIR3DL1-HLA-Bw4 had a significantly higher SVR rate (68% [38/56] vs. 31% [18/59]; P = 0.000062 [Pc = 0.0005]; OR = 4.81, 95% CI = 2.19 - 10.58). The frequency of a non-SVR was significantly higher in patients with the IL28B non-TT genotype both with and without

#### Table 3. Logistic regression analysis of variables contributing to a sustained virological response to pegylated interferon and ribavirin.

| Factor                      | Odds ratio | 95% confidence interval | P       |
|-----------------------------|------------|-------------------------|---------|
| IL28B TT genotype          | 6.87       | 2.62 - 18.01             | 0.0009  |
| KIR2DL2-HLA-C1              | 0.24       | 0.08 - 0.75              | 0.014   |
| White blood cells ≥ 4410μL  | 3.32       | 1.35 - 8.16              | 0.009   |
| KIR3DL1-HLA-Bw4             | 3.32       | 1.37 - 8.05              | 0.008   |

Only variables achieving statistical significance (P < 0.05) in multivariate logistic regression analysis are shown. doi: 10.1371/journal.pone.0083381.t003

KIR2DL2-HLA-C1 (14% [8/59] vs. 0% [0/8]; P = 0.013 [Pc = 0.1] and 41% [24/59] vs. 21% [12/56]; P = 0.026 [Pc = 0.21]; OR = 4.00, 95% CI = 1.17 - 0.91, respectively). The ability to predict an SVR by IL28B genotype and KIR3DL1-HLA-Bw4 and KIR2DL2-HLA-C1 was next evaluated. Corresponding values for sensitivity, specificity, PPV, and NPV are listed in Table S1 in File S1. A combination of the IL28B TT genotype and KIR3DL1-HLA-Bw4 demonstrated high predictive specificity (78%), as did the combination of IL28B TT genotype and KIR2DL2-HLA-C1 (86%).

Lastly, we analyzed combinations of the three factors of IL28B genotype, KIR3DL1-HLA-Bw4, and KIR2DL2-HLA-C1 for prediction of treatment outcome (Table S2 in File S1). The frequencies of IL28B TT, KIR2DL2-HLA-C1-negative, with and without KIR3DL1-HLA-Bw4 were significantly higher among responders (38% [21/56] vs. 19% [11/59]; P = 0.024 [Pc = 0.29]; OR = 2.62, 95% CI = 1.12 - 6.12 and 30% [17/56] vs. 12% [7/59]; P = 0.015 [Pc = 0.18]; OR = 3.24, 95% CI = 1.22 - 8.57, respectively).
Discussion

The present study examined HLA, KIR, and IL28B gene variant associations with an SVR following PEG-IFN and ribavirin therapy in Japanese patients with chronic hepatitis C. We found a significant association of HLA-Bw alleles with treatment outcome, although the frequency of HLA-C alleles did not differ significantly between responders and non-responders. Functional analyses have demonstrated that NK cells in HLA-C1C1 subjects exhibit a more rapid and stronger antiviral response that those in HLA-C2C2 subjects due to differing responses of HLA-C-inhibited NK subsets[33]. HLA-C2C2 homozygosity is strongly associated with treatment failure in HCV patients of European ancestry [11,22], but we could not assess its role in our study because this genotype was found in only 1 of 115 patients.

We uncovered a significant association between the presence of KIR2DL2 or KIR2DS2 and lower SVR rates. Several reports have shown that KIR2DL3-HLAC1 in Caucasians [11,22] and KIR2DL5 in Brazilians [34] are associated with treatment outcome of antiviral therapy. Since our results showed no such statistical significances, these conflicting interpretations may reflect differences in patient selection, genetic background, sample size, and/or treatment regimen. Further studies are required to clarify this discrepancy in the Japanese population.

A study by Dring et al. examined KIR haplotypes in patients with HCV infection and showed that a centromeric KIR haplotype was increased in chronic HCV infection as compared with resolved cases [20]. We therefore determined KIR haplotypes and Cen-A/B and Tel-A/B in our patients as well, and found an interesting association between Cen-A/A and an SVR to antiviral therapy (P = 0.015; OR 3.37). Since Cen-A/B is determined by KIR2DL3 and KIR2DS2 and/or KIR2DL2, this finding is consistent with our results demonstrating a relationship between KIR2DS2 and KIR2DL2 genotypes and treatment failure.

The most significant finding in this study was the association between KIR-HLA receptor-ligand pairings and treatment outcome in chronic hepatitis C. Among the inhibitory KIR-HLA receptor-ligand pairs, patients with KIR3DL1-HLA-Bw4 exhibited a significantly higher SVR rate when compared to those without this pair (P = 0.03; OR 2.29). Conversely, virologic clearance in patients with KIR2DL2-HLA-C1 was significantly lower than those without (P = 0.015; OR = 0.30). Stratification analysis of the 4 groups of KIR3DL1-HLA-Bw4 (presence or absence) and KIR2DL2-HLA-C1 (presence or absence) revealed a higher frequency of responders with KIR3DL1-HLA-Bw4 presence, KIR2DL2-HLA-C1 absence compared with those possessing KIR2DL2-HLA-C1 presence, KIR3DL1-HLA-Bw4 absence (62% vs. 9%; P = 0.0044; OR = 16.32). When these KIR-HLA pairs were both either positive or negative, SVR rates were similar at 42% and 45%, respectively. Together with the results of logistic regression analysis, we clearly showed that KIR3DL1-HLA-Bw4 was positively associated with an SVR (OR = 3.32) and that KIR2DL2-HLA-C1 had a negative association (OR = 0.24) with treatment outcome. As almost one half of the Japanese population have the functional KIR3DL1-HLA-Bw4 combination, this inhibitory receptor-ligand interaction is potentially important in understanding NK cell diversification. The NK-cell surface expression of KIR3DL1 is higher in individuals having Bw4 than in those lacking it [35]. Therefore, these cells might be more weakly controlled by inhibitory signals than other NK cells, more easily activated by viral infection, and more readily promoted for cytolysis and IFN-gamma production.

This study confirmed that the IL28B TT genotype is a strong predictor of an SVR in Japanese patients[18,32]. Furthermore, SVR frequencies were positively correlated with a combination of the IL28B TT genotype and KIR3DL1-HLA-Bw4 (P = 0.0019) and negatively associated with the IL28B TT genotype and KIR2DL2-HLA-C1 (P = 0.0067). These combinations were also highly specific for virologic response prediction. In light of these findings, patients with poor expected treatment outcome may be advised to wait for the use of combinations of direct acting antiviral agents[36]. Akuta et al. reported that a combination of amino acid substitutions in the core region of HCV and IL28B genotype was a useful predictor of PEG-IFN, ribavirin, and telaprevir therapy results in Japan[37]. Since we could not collect sera before treatment for all patients, we were not able to assess the effect of amino acid substitutions in the HCV core region. Furthermore, interferon-free combinations of direct-acting antiviral agents have become an area of considerable clinical interest. Chu et al. have reported that IL28B genotype appears to affect early viral kinetics in patients with chronic hepatitis C receiving interferon-free treatment [38]. Recently, two groups have discovered IFN lambda 4 (IFNL4), a new gene that may account for associations of spontaneous and IFN-based treatment clearance of HCV [39,40]. The IFN-λ 4 protein is generated by individuals who carry the ∆G allele of the ss469415590 variant, and the presence of this protein is strongly associated with impaired clearance of HCV. Linkage disequilibrium is strong between the IFNL4-∆G allele and the unfavorable rs12979860-T allele (IL28B) in subjects of European or Asian ancestry, whereas this linkage disequilibrium is moderate in individuals of African ancestry [39]. We have confirmed that the linkage disequilibrium between the IFNL4-∆G allele and IL28B SNP (rs8099917) is high and that the IFNL4-∆G allele is strongly associated with treatment failure of PEG-IFN and ribavirin therapy in patients with Japanese chronic hepatitis C [41]. Hence, the clinical impacts of HLA-KIR genetic variants, IL28B genotype, and the IFNL4 allele should be explored.

In conclusion, the present study showed significant associations of KIR3DL1-HLA-Bw4, KIR2DL2-HLA-C1, and IL28B combinations with an SVR to PEG-IFN and ribavirin therapy in Japanese patients with genotype 1 HCV. The clinical significance of IL28B genotyping combined with HLA/KIR pairs to predict treatment outcome warrants further validation for triple therapy.

Supporting Information

File S1. Table S1, Sensitivity, specificity, and predictive values of IL28B TT genotype and KIR3DL1/HLA-Bw4 or
KIR2DL2/HLA-C1 for a sustained virological response in 115 patients with chronic hepatitis C. Data are expressed as % (n). PPV, positive predictive value; NPV, negative predictive value. Table S2. Frequency of IL28B genotype and KIR3DL1/HLA-Bw4 and KIR2DL2/HLA-C1 combinations in 56 patients with a sustained virological response (SVR) and 59 patients with a non-SVR to pegylated interferon and ribavirin therapy of chronic hepatitis C. Data are expressed as n (%).

(DOC)

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