Association of IL28B gene variations with mathematical modeling of viral kinetics in chronic hepatitis C patients with IFN plus ribavirin therapy

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Asian patients with chronic hepatitis C (CHC) are known to have better virological responses to pegylated (Peg) IFN-based therapy than Western patients. Although IL28B gene polymorphisms may contribute to this difference, whether favorable hepatitis C virus (HCV) kinetics during treatment plays a role remains unclear. We enrolled 145 consecutive Taiwanese patients with CHC receiving Peg-IFN α-2a plus ribavirin for the study. Blood samples were taken more frequently at defined intervals in the first 3 d. Peg-IFN was administered at week 1. It was then administered weekly in combination with daily ribavirin for 24 or 48 wk. A mathematical model fitted to the observed HCV kinetics was constructed, which could interpret the transient HCV titer elevation after Peg-IFN treatment. The results demonstrated a comparable viral clearance rate ($c = 3.45 \pm 3.73$) (day^{-1}, mean ± SD) but lower daily viral production rate ($P = 10^{6}$–10^{12}) in our patients than those reported previously in Western patients. Of 110 patients with a sustained virological response (SVR), 47 (43%) had a transient elevation of viral titer within 12 h (proportion of 12 h/3 d: 44% in non-SVR vs. 70% in SVR; $P = 0.029$). Among 91 patients with available rs8099917 data, patients with the TT genotype had an early surge of viral titer after therapy and a higher SVR and viral clearance rate than those with the GT genotype. In conclusion, Taiwanese patients with CHC receiving Peg-IFN plus ribavirin therapy have a lower daily viral production rate than Western patients, and the rs8099917 TT genotype may contribute to the increased viral clearance rate and better virological responses in these patients.

Hepatitis C virus (HCV) infection is the major etiology of chronic liver disease, liver cirrhosis, and hepatocellular carcinoma (1, 2). According to the estimate from the World Health Organization, there are more than 180 million chronic HCV-infected persons worldwide (1–3); hence, effective treatment of chronic hepatitis C (CHC) is important. Nevertheless, the current standard of care for CHC using pegylated (Peg) IFN plus ribavirin is expensive, is effective in only a certain proportion of patients who have CHC, and has many unpleasant adverse effects (4, 5). Therefore, identifying predictive factors of therapeutical response in patients with CHC is important.

Several factors have been linked to the therapeutical response of patients who have CHC, including viral factors (6–11), host factors (12–15), metabolic factors (16–18), histological factors (19), types of regimen (4), and duration of infection (20). Among these factors, viral kinetics following antiviral therapy has become widely accepted in both clinical trials and daily practice (21) and increasingly recognized as the most outweighing predictor of sustained virological response (SVR) to IFN-based therapy (22). Using mathematical models of hepatitis C viral kinetics may further clarify the mechanisms of antiviral therapy, the evolution of resistant viral strains, and the length of time necessary to eradicate the infection (23, 24). Several issues still deserve attention, however. First, the frequently used Neumann's model is based on the daily dosing of standard IFN (25), which is different from the current standard of care, Peg-IFN plus ribavirin; whether this mathematical model remains feasible needs further examination (26). Second, hepatitis C viral load rebounds are frequently observed toward the end of the weekly Peg-IFN dosing and will either underestimate or overestimate the effectiveness as well as the infected cell loss rate in a given patient (27, 28). Third, the quantitative assays of HCV RNA to construct models are limited by their detection thresholds (29). Finally, most currently used models are based on the data of Western patients with CHC; whether they are applicable to Asian patients remains largely unknown.

Recently, it has been documented that there was a strong association of two SNPs 3–8 kb upstream of the IL28B gene encoding IFN-λ-3, rs8099917 and rs12979860, with treatment outcomes of Peg-IFN α-2a plus ribavirin therapy (30–34). These variations in the IL28B gene correlated well with natural clearance of HCV and with SVR, and they may explain the difference in response rates among African-Americans, Europeans, and Asians. According to the International HapMap Project, the Han Chinese people have a higher prevalence of the T allele and TT genotype than subjects of European ancestry (Han Chinese vs. European, T/G allele: 93.5/6.5 vs. 85/15; TT/GT genotype: 87/13 vs. 73/25). The influence of IL28B gene polymorphisms on viral kinetics in patients with CHC who are receiving Peg-IFN plus ribavirin therapy remains unclear, however, and thus deserves further study. To this end, we enrolled 145 consecutive treatment-naïve Taiwanese patients with CHC receiving the combination therapy of Peg-IFN plus ribavirin to construct a unique viral kinetic model and further evaluated the impact of rs8099917 genotypes on these patients by using this unique mathematical model.

Results

Baseline Characteristics of Patients. A total of 145 patients with CHC were enrolled for this study of viral kinetics. Among them,

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91 with available SNP (rs8099917) data were studied to evaluate the impact of IL28B gene polymorphisms on viral kinetics. The baseline characteristics and virological responses were comparable between patients with and without SNP (rs8099917) data (Table S1). Of the 91 patients with available SNP (rs8099917) data, 46 (50.6%) had HCV genotype 1 infection and the remaining 45 (49.5%) had HCV genotype 2 infection.

Transient Viral Titer Elevation and SVR. The trends of successional viral titers in patients with CHC after the first dose of Peg-IFN were different between patients with and without SVR. Although viral titer declined in most patients, a transient elevation of HCV titer after Peg-IFN treatment was significantly associated with a higher SVR in patients with CHC (Figs. 1 and 2). Of 110 patients who achieved an SVR, 67 (61%) had a transient elevation of viral titer during the first 3 d, with 47 (43%) having a transient elevation of viral titer within 12 h after the first dose of Peg-IFN (Table 1).

Viral Kinetic Parameters. To estimate HCV viral kinetic parameters, the viral load, V, was fit to the serial viral loads of the first 3 d in 145 Taiwanese patients with CHC receiving Peg-IFN α-2a plus ribavirin by a nonlinear least squares method. Models with four or three parameters were used to estimate the clearance and production rate of virions. Because only the three-parameter model could model the important observation of early transient HCV titer elevation after Peg-IFN treatment, we analyzed these data under the assumption of $c = \delta$, where c is the clearance rate, and found that Taiwanese patients with CHC had a comparable clearance rate but a lower production rate than in the previously reported Western patients (Fig. S1 and Table S2).

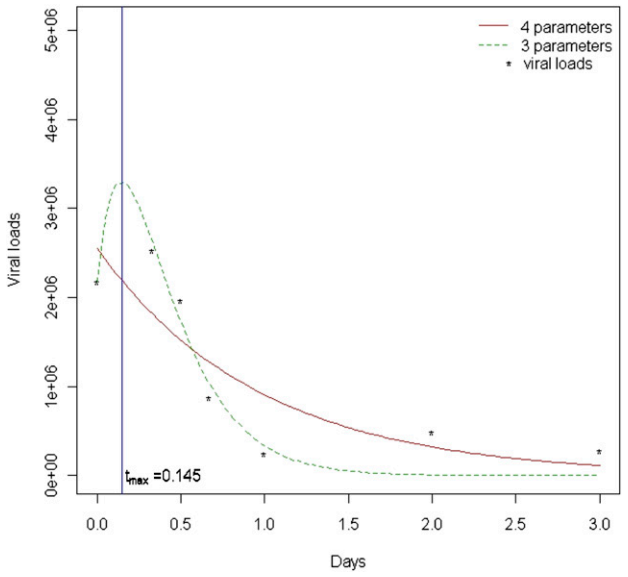

Fig. 1. Estimated time of maximal viral load for a patient with viral titer elevation. In the figure, the viral loads are fitted by the two models that have three and four parameters; obviously, the three-parameter model (dashed green line) is better than the other one in the early stage. Only the three-parameter model may interpret the viral surge that is also noted in the other study (25). The observed data also show that the occurrence of viral elevation is significantly associated with SVR. Because frequent measurement is impractical, the viral elevation may be missed. The three-parameter model may not only model the viral elevation but estimate the time when the maximal viral load occurs if elevation exists. Thus, the maximal viral load and its time of occurrence may be useful markers to predict the response to treatment. To estimate HCV viral kinetic parameters, the V(t) from the analytical solution (Eq. **4**) is fit to the viral load data of the first 3 d individually for each patient by a nonlinear least squares method using R (version 2.10.0), simultaneously fitting K1′, K2′, and s′ (assuming t0 = 0). Here, we show the result by fitting the serial viral loads of the first 3 d from this patient who finally achieved an SVR. For the viral load, $V = (K_1' t + K_2')e^{s't}$. For estimated time of the maximal viral load, $t_{max} = -1/s' - K'_2/K'_1 = 1/c - K'_2/K'_1$, V is the viral load, t = 0 is the time of the first injection, and t_{max} is the estimated time of maximal viral load for patients with viral titer elevation.

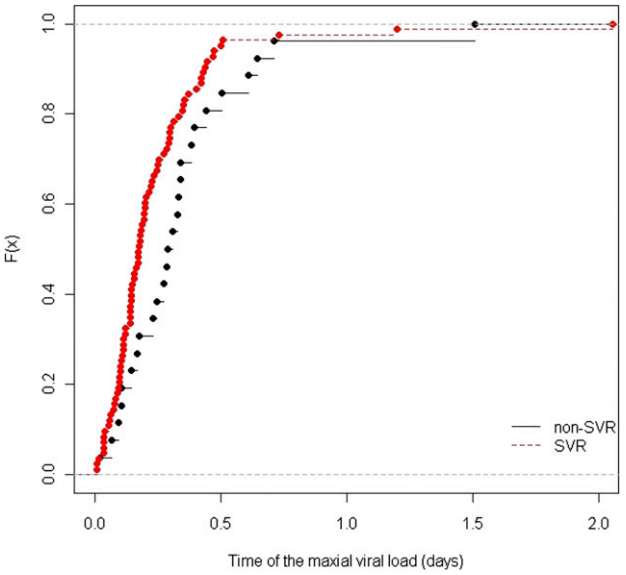

Fig. 2. Empirical cumulative density functions of the estimated time of maximal viral loads for the patients with viral titer elevation. The estimated time of maximal viral load of the SVR for patients with elevation is significantly shorter than that for patients without an SVR [red circle for patients with an SVR and black circle for patients without an SVR (non-SVR); $P = 0.0095$ and $P = 0.0541$ by a one-sided Kolmogorov–Smirnov test and *t* test, respectively]. The results show that an earlier elevation of viral load is associated with a higher likelihood of SVR. F(x) indicates empirical cumulative density functions of the estimated time of maximal viral load for the patients with viral titer elevation.

Table 1. Time of transient hepatitis C viral titer elevation in patients with and without an SVR

	No SVR, no. (%)	SVR, no. (%)
Time after first dose of Peg-IFN, d		
0.33	7 (28)	25 (37.3)
0.5	4 (16)	22 (32.8)
0.67	13 (52)	14 (20.9)
1.0	0 (0)	3 (4.5)
1.5	0 (0)	1 (1.4)
2.0	1 (4)	1 (1.4)
3.0	0 (0)	1 (1.4)
Time of viral titer elevation		
In 12 h	11 (44)	47 (70)
From 12 h to day 3	14 (56)	20 (30)

Data are shown by case number (proportion in %). Patients with transiently elevated hepatitis C viral titers were those whose hepatitis C viral titers during the first 3 d of treatment were higher than baseline titers, and 92 patients had transient elevated hepatitis C viral titers before the decline of viral load. Patients with transiently elevated hepatitis C viral titers within 12 h after Peg-IFN treatment were those whose hepatitis C viral titers during the first 12 h of treatment were higher than baseline titers. SVR was defined as undetectable serum HCV RNA 24 wk after cessation of treatment, and we used 34 IU/mL as the undetectable level of serum HCV RNA. During the first 3 d, no SVR vs. SVR (27.2% vs. 72.8%), $P = 0.075$; within the first 12 h, no SVR vs. SVR (44% vs. 70%), $P = 0.029$ (two-sided), $P = 0.020$ (one-sided).

Association of the SNP (rs8099917) and Virological Responses. The prevalence rates of the T allele and TT genotype of our patients were similar to those in the reports from the International HapMap Project. Of 91 patients with available SNP (rs8099917) data, 77 (84.6%) had a TT genotype and 14 (15.4%) had a GT genotype but none had a GG genotype. Compared with patients with a GT genotype, those with a TT genotype had higher SVR rates [TT genotype vs. GT genotype: 67 (87%) vs. 7 (50%); $P = 0.0037$] (Table 2) and rapid virological response (RVR) rates [TT genotype vs. GT genotype: 64 (83.1%) vs. 8 (57.1%); $P = 0.028$] but comparable complete early virological response (c-EVR) rates [TT genotype vs. GT genotype: 75 (97.4) vs. 13 (92.9); $P = 0.381$]. To evaluate the impact of different rs8099917 genotypes on viral kinetics, we estimated and compared the viral production rate as well as the clearance rate between patients with TT and GT genotypes. Patients with a TT genotype had a significantly higher viral clearance rate ($P = 0.0234$) and tended to have an earlier surge of viral titer after Peg-IFN treatment ($P = 0.0502$) than those with a GT genotype (numbers with hepatitis C viral titer elevation in 12 h/numbers in 3 d; TT genotype vs. GT genotype: 38/50 vs. 4/10; $P = 0.023$) (Table 2 and Fig. S2). Moreover, we constructed different models to examine the interaction and association of various factors with SVR; identified TT genotypes and virion production rate, rather than HCV genotype or virion clearance rate, were significant predictors of SVR (Table 3 and Fig. S3). HCV genotype is known to be a predictor of SVR; however, it serves as a latent factor of viral kinetics, especially for virion production rate ($P = 0.0006$) (Table S3).

Discussion

In this study, we hypothesized that patients with CHC who have different SNPs in IL28B may have different hepatitis C viral kinetics during Peg-IFN plus ribavirin treatment and that, through this linkage, patients with CHC who have different IL28B SNPs may have different therapeutical responses. To this end, we constructed a unique hepatitis C viral kinetic model to fit a large cohort of patients with CHC. Compared with viral kinetic data observed in Western patients with CHC treated with IFN-based therapy, our results showed a comparable viral clearance rate but lower daily viral production rate in Taiwanese patients with CHC receiving Peg-IFN α-2a plus ribavirin therapy. In addition, we found a significant association of transient hepatitis C viral titer elevation after therapy with better virological responses. We also examined the impact of a recently identified SNP (rs8099917) on viral kinetics after IFN-based therapy. Our data not only showed a prevalence rate of the TT genotype comparable to that in the reports of the International HapMap Project but indicated that the TT genotype was significantly associated with better RVR and SVR rates.

First, we constructed a viral acceleration model to explain the viral dynamics of patients with CHC receiving Peg-IFN plus ribavirin treatment under the assumption of target cells, T, remaining at their baseline value for 2 wk. With the same assumption and a higher order differential equation to construct the model, the flexibility, applicability, and fit of the current model would be better than those of previous models. In addition, taking advantage of a larger cohort of patients with CHC for viral kinetic observation and analyses, we not only found that transient hepatitis C viral titer elevation after Peg-IFN treatment was common in patients with better virological responses but estimated the viral kinetic parameters in Taiwanese patients with CHC. Although the phenomenon of transient hepatitis C viral titer elevation after IFN-based treatment has been found before (26, 28, 35, 36), the clinical significance of this observation remains unclear. In the beginning, we used different models with four or three parameters to model hepatitis C viral titers and found that both models fitted the biphasic changes but that only the three-parameter model could model the early transient hepatitis C viral titer elevation after Peg-IFN treatment. The three-parameter model was only valid under the condition that $(c - \delta)^2 + 4(1 - \varepsilon)p(1 - \eta)\beta T = 0$; thus, $c = \delta$ and $\varepsilon = 1$ or $\eta = 1$ should exist simultaneously. By differentiating V and comparing it with Eq. **3**, only the condition $c = \delta$ and $\eta = 1$ was valid, suggesting a complete block on de novo HCV infection in patients with an earlier surge of hepatitis C viral titer during

Table 2. rs8099917 TT genotype is associated with higher viral clearance rate, early surge of viral titer after therapy, and higher SVR rate

	Rs8099917		
	TT genotype	GT genotype	*P* value
SVR	67	7	0.0037*
No SVR	10	7	
Clearance rate	3.56 ± 3.51	3.79 ± 7.21	0.9070†
			0.0234‡
Clearance rate (excluding extreme value, >20)	3.22 ± 2.02	1.92 ± 1.75	0.0259†
			0.0114‡
Log_{10} (production rate)	10.74 ± 1.17	10.34 ± 1.07	0.2210†
			0.2315‡
Time of viral titer elevation, d			0.0502*
0.33	20	3	
0.5	18	1	
0.67	10	4	
1.0	0	1	
1.5	1	0	
2.0	0	1	
3.0	1	0	
Estimated time of maximal viral load with elevation, d	0.21 ± 0.14	0.40 ± 0.61	0.36†

Data are shown by mean ± SD or case number. SVR was defined as undetectable serum HCV RNA 24 wk after discontinuation of treatment, and we used 34 IU/mL as the undetectable level of serum HCV RNA.
*Fisher's exact test.
†Student's *t* test.
‡Kolmogorov–Smirnov test.

Table 3. Multivariate analysis identifying factors associated with SVR in 91 patients with CHC receiving the combination therapy

	Model 1		Model 2		Model 3	
	Coefficient	*P* value	Coefficient	*P* value	Coefficient	*P* value
Intercept	12.4662	0.0133	12.4944	0.0130	14.5007	0.0014
Clearance rate	0.3827	0.1098	0.3897	0.0975	0.4541	0.0453
Log_{10} (production rate)	−1.0786	0.0179	−1.0840	0.0171	−1.2576	0.0024
rs8099917	−2.6815	0.0144	−2.5965	0.0045	−2.6630	0.0029
HCV genotype	0.6936	0.4423	0.7511	0.3591		
rs8099917 × HCV genotype	0.2548	0.8856				
AIC	72.194		70.215		69.097	

SVR was defined as undetectable serum HCV RNA 24 wk after discontinuation of treatment, and we used 34 IU/mL as the undetectable HCV RNA level. The logistic regressions for SVR among different models are shown in this table. Multivariate analysis with logistic regression modeling was performed to examine the association and interaction between various factors and SVR. SVR was used as the dependent variable, and SNP rs8099917, HCV genotype, virion clearance rate, production rate, and interaction term of SNP rs8099917 and HCV genotype were used as independent variables in model 1; SNP rs8099917, HCV genotype, virion clearance rate, and production rate were used as independent variables in model 2; and virion clearance rate, production rate, and SNP rs8099917 were used as independent variables in model 3. According to model selection by the Akaike Information Criterion (AIC), model 3 would be the most appropriate model for SVR. The patients with a higher clearance rate, lower production rate, and TT genotype would be more likely to achieve an SVR. Otherwise, the virus genotype could be a latent factor influencing the kinetic parameters, or its effect could be shared by the kinetic parameters.

IFN-based therapy. In addition, previous studies on HCV kinetics after liver transplantation have indicated that the liver is the major organ to clear the virus (37). Thus, if we could block de novo HCV infection, the virion clearance rate in the liver would be substantially increased. This speculation lends strong support to our observation that an earlier surge of viral titer led to a better virological response in patients with CHC receiving the combination therapy. Moreover, if a given patient fails to show an earlier surge of viral titer after receiving Peg-IFN plus ribavirin treatment, it indicates that the de novo HCV infection is only partially blocked and might imply a poor virological response during the earlier viral kinetic phase. Whether the patient could achieve an SVR remains unknown, however, because of other factors that might operate during the later phase of viral kinetics and offset this earlier drawback.

Although our estimates on virion clearance rate and production rate were lower than those reported by Neumann et al. (25), the clearance rate was comparable to the estimate by Herrmann et al. (26). This discrepancy may be explained by the different pharmacokinetics between regular IFN and Peg-IFN. Moreover, because virus production was calculated for each patient by multiplying the initial viral load, the clearance rate, and a volume factor of 13,360 mL in extracellular fluid for a person with a standard body weight of 70 kg as previously described (38), lower virion clearance during Peg-IFN treatment might be transformed to a lower virion production rate, regardless of the initial viral load. Conversely, the clearance rate might also be affected by the initial viral load; thus, the clearance rate and production rate may not be correlated.

IL28A, IL28B, and IL29 (IFN-λ II, III, and I, respectively) are newly identified IFNs (39) with similarities to type I IFNs in terms of biological activity and mechanism of action, but they differ structurally as well as genetically (40) and are known to have antiviral effects against hepatitis B virus and HCV (41). Recent studies have demonstrated an association of genetic variations in IL28B with the expression levels of IL28B (plus IL28A) in peripheral blood mononuclear cells (32, 33) as well as with the effect on response to IFN-based therapy. In our study, we further examined the impact of an SNP (rs8099917) on viral kinetics in patients with CHC receiving IFN-α therapy and found that the TT genotype was associated with a better SVR, RVR, and virion clearance rate, as well as with an earlier surge of viral titer after Peg-IFN treatment, but not with a better early virological response (EVR). These facts indicated that genetic variations of IL28B may influence the earlier phase of viral kinetics after IFN-based treatment. Conversely, although HCV genotype has long been known to be a predictor of SVR, it serves as a latent factor of viral kinetics, especially for virion production rate (the log_{10} virion production rate of genotype 1 vs. genotype 2: 11.03 ± 0.96 vs. 10.17 ± 1.21; $P = 0.0006$) (Table S3). Thus, the impact of HCV genotype on SVR in the regression models becomes less significant, and viral kinetic parameters are shown to be better than HCV genotype in the prediction of SVR. Therefore, our data suggested that the TT genotype and virion production rate, rather than HCV genotype 1, HCV genotype 2, or the virion clearance rate, were factors predictive of SVR and strongly supported the concept that viral kinetics or on-treatment HCV RNA levels are the important predictors of SVR in patients with CHC receiving IFN-based therapy (22). Because Han Chinese people are known to have a higher prevalence of the T allele than subjects of European ancestry (International HapMap Project), the different prevalence rates of specific genotypes may partly explain the better virological response in Asian patients with CHC treated with the combination therapy.

In conclusion, Taiwanese patients with CHC who received Peg-IFN α-2a plus ribavirin therapy have a lower daily viral production rate than Western patients, and the increased viral clearance and better virologic response rate may be attributable to the rs8099917 TT genotype. In addition, transient hepatitis C viral titer elevation after IFN-based treatment may predict a better virological response, which can be explained by a complete block on de novo HCV infection of hepatocytes. Further studies are needed to explore the mechanisms involved in the association of specific IL28B gene variations with treatment-induced HCV clearance (additional information is provided in *SI Materials and Methods*).

Materials and Methods

Study Population. In a previous prospective study of factors affecting early viral load decline during treatment with combination therapy, 145 consecutive patients with CHC were enrolled from the gastroenterological clinics of the National Taiwan University Hospital and its Yun-Lin Branch, as previously reported (24). In brief, chronic HCV infection was defined as the positivity of both anti-HCV and serum HCV RNA for more than 6 mo. All patients had

available histological data and were naïve to IFN treatment and other experimental antiviral or immunosuppressive therapy. They had serum alanine aminotransferase levels at least twice the upper limit of normal on two occasions within the previous 6 mo. None of them were positive for hepatitis B surface antigen or HIV antibody or had a known history or serological evidence of autoimmune liver disease, inheritable disorders like hemochromatosis or Wilson's disease, renal insufficiency, malignancy, daily alcohol consumption greater than 20 g, or active drug abuse.

HCV genotype 1 patients received 180 μg of Peg-IFN α-2a plus ribavirin for 48 wk, and genotype 2 patients received 180 μg of Peg-IFN α-2a plus ribavirin for 24 wk; all patients were followed for 24 wk after discontinuation of treatment. One dose (180 μg) of Peg-IFN α-2a was administered s.c. at the beginning of the study (day 0). Then, 180 μg of Peg-IFN α-2a plus ribavirin was administered from week 1 to week 24 or week 48. The dosage of oral ribavirin was adjusted according to body weight (1,000 mg for a weight of 75 kg or less and 1,200 mg for a weight greater than 75 kg). Scheduled blood sampling was performed for HCV RNA detection and quantification. Serum HCV RNA levels were quantified before the first dose (0 h); 4 h, 8 h, and 12 h after first dose on day 0; daily for 3 consecutive days (days 1–3) during week 0, and then at week 4, week 12, week 24, and every 24 wk until the end of the follow-up period.

From January 2007 to December 2008, patients who participated in a previous study were invited to participate in an additional host genomic survey, and 91 of them agreed to participate in this survey.

Ethical Considerations. The study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the Ethical Committee of the National Taiwan University Hospital. All patients gave informed consent before enrollment, and their viral parameters and biochemical, serological, and anthropometric data were recorded at enrollment. The genomic DNA of 91 patients who agreed to participate in the host genomic survey was obtained after they signed the informed consent form (*SI Materials and Methods*).

Definitions of Treatment Response. The virological response to therapy was based on serum HCV RNA levels (42–44). RVR was defined as an undetectable serum HCV RNA level at day 28. EVR was defined as an undetectable serum HCV RNA level or at least 2 log decrease of baseline HCV RNA level at week 12. c-EVR was defined as an undetectable serum HCV RNA level at week 12 of therapy, and partial EVR was defined as at least 2 log reduction of serum HCV RNA level from baseline to week 12 of therapy. Sustained virological responders were defined as those having an undetectable serum HCV RNA level 24 wk after cessation of the treatment (24, 45).

Mathematical Modeling of Viral Kinetics. The data were analyzed using a previously published mathematical model for the effect of IFN-α on HCV dynamics (25, 46). The model differential equations are as follows:

$$dT/dt = s - dT - (1-\eta)\beta VT + qI \quad [1]$$

$$dI/dt = (1-\eta)\beta VT - kI - qI \quad [2]$$

$$dV/dt = (1-\varepsilon)pI - cV \quad [3]$$

In this model, T represents the number of target cells, I represents the number of productively infected cells, and V is the viral load. Target cells are produced at rate s and die at death rate constant d. Cells become productively infected at de novo infection rate constant β and, once infected, may be killed at rate constant k or become nonproductive at rate constant q. HCV virions are produced by infected cells at an average rate of p virions per cell per day and are cleared at a clearance rate constant c. In this model, IFN-α is assumed to reduce virion production by infected cells by a fraction $(1-\varepsilon)$ but could also reduce de novo infection of target cells by a factor $(1-\eta)$. Before IFN-α therapy, $\varepsilon = \eta = 0$. At t = t0, in which t = 0 is the time of the first injection and t0 is a delay possibly because of a pharmacokinetic lag, IFN-α is assumed to block virus production ($0 \le \varepsilon \le 1$) and to block de novo infection ($0 \le \eta \le 1$). Assuming that the number of target cells does not change significantly over the course of the first weeks of treatment [T(t) = T0], it is not possible to uncouple the effects of killing infected cells (k) or making them nonproductive (q), and one needs to use a combined parameter δ = k + q depicting the total loss rate of productively infected cells. By differentiating the change rate of viral load (dV/dt) and assuming that all the parameters are constant except for viral load and number of productively infected cells, we can obtain the acceleration of viral load according to Eqs. **2** and **3**:

$$d^2V/dt^2 = -(c+\delta)dV/dt - [\delta c - (1-\varepsilon)p(1-\eta)\beta T]V \quad [4]$$

For a reasonable assumption that the number of target cells, T, will remain constant during the first 2 wk after the first injection of IFN-α, there will be two possible analytical solutions for the second-order differential equation:

$$i)\ V = K_1 e^{s1t} + K_2 e^{s2t}$$

$$ii)\ V = (K_1't + K_2')e^{s't}$$

For (i) :

$$S1 = -0.5(c+\delta) - 0.5[(c-\delta)^2 + 4(1-\varepsilon)p(1-\eta)\beta T]^{1/2}$$

$$S2 = -0.5(c+\delta) + 0.5[(c-\delta)^2 + 4(1-\varepsilon)p(1-\eta)\beta T]^{1/2}$$

The S1 and S2 can be approximated by the −c and −δ because ε and η are close to 1.

$V = (K_1't + K_2')e^{s't}$ will be valid only under the condition that $(c-\delta)^2 + 4(1-\varepsilon)p(1-\eta)\beta T = 0$; thus, if (*ii*) is valid, c = δ and ε = 1 or η = 1 should simultaneously exist and, finally, $s' = -0.5(c+\delta) = -c = -\delta$. By differentiating V and comparing it with Eq. **3**, only the condition c = δ and η = 1 is valid. Otherwise, only the elevation of the virus loads can be interpreted under the condition; thus, we only fit this model.

Nonlinear Data Fitting. The estimation by fitting the log of viral loads is not included for further analyses because it will eliminate the immediate slightly increasing effect of the viral loads after the first dose. To estimate HCV viral kinetic parameters, the V(t) from the analytical solution (Eq. **4**) is fit to the viral load data of the first 3 d individually for each patient by a nonlinear least squares method using R (version 2.10.0; The R Foundation for Statistical Computing), fitting K1′, K2′, and s′ (assuming t0 = 0) simultaneously.

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