

# CLINICAL ADVANCES IN LIVER, PANCREAS, AND BILIARY TRACT

## Replicated Association Between an *IL28B* Gene Variant and a Sustained Response to Pegylated Interferon and Ribavirin

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**BACKGROUND & AIMS:** Patients with chronic hepatitis C virus (HCV) infections are treated with pegylated interferon and ribavirin (PEG-IFN/RBV), which is effective in less than 50% of those infected with HCV genotype 1. Genome-wide association studies have linked response to PEG-IFN/RBV with common single nucleotide polymorphisms in the vicinity of interferon (*IFN*)- $\lambda$  genes on chromosome 19. We investigated the association between the polymorphism rs12979860 and treatment response in a diverse cohort of chronic HCV patients. **METHODS:** A cross-sectional study of 1021 consecutive patients enrolled in the Duke Hepatology Clinic Research Database and Biorepository. We analyzed DNA, clinical and demographic data, along with validated data of the response of 231 subjects to PEG-IFN/RBV. The study included Caucasians ( $n = 178$ ), African Americans ( $n = 53$ ), and HCV genotypes 1 ( $n = 186$ ) and 2/3 ( $n = 45$ ). The rs12979860 genotype was tested for an association with sustained virologic response, defined as undetectable levels of HCV RNA 24 weeks after treatment ended. **RESULTS:** The rs12979860 CC genotype (found in ~40% of Caucasians) predicted a sustained virologic response to therapy among Caucasians (odds ratio, 5.79; 95% confidence interval, 2.67–12.57;  $P = 9.0 \times 10^{-6}$ ), independent of HCV genotype and other covariates. Rs12979860 CC predicted a sustained response with 78% specificity and 65% sensitivity in patients infected with HCV genotype 1). **CONCLUSIONS: rs12979860 genotype is a significant independent predictor of response to PEG-IFN/RBV in patients with chronic HCV infection; tests for this genotype might be used to determine the best course of treatment for patients considering antiviral therapy.**

*Keywords:* Pharmacogenetic; Interferon- $\lambda$ ; Viral Load; Single Nucleotide Polymorphism.

people are thought to be infected, representing 1.6% of the population.<sup>2,3</sup> Chronic HCV infection can lead to progressive liver disease, resulting in cirrhosis and complications including decompensated liver disease and hepatocellular carcinoma leading to the need for transplantation.<sup>2,3</sup> The current standard-of-care treatment for suitable patients with chronic HCV infection consists of pegylated interferon alfa 2a or 2b (PEG-IFN) given by injection in combination with oral ribavirin (RBV), for 24 or 48 weeks, dependent on HCV genotype. This treatment is not only costly, but is associated with significant side effects resulting in reduced compliance and fewer patients completing treatment. Only about half of treated subjects achieve a sustained virologic response (SVR).<sup>4-6</sup> For all these reasons, decisions regarding treatment with current standard-of-care IFN-based therapy are complex and based on the balanced assessment of host and viral determinants that aid in predicting virologic response. HCV genotype, in particular, is used in making treatment decisions: patients with HCV genotype 2/3 have a relatively high rate of SVR (70%–80%) with 24 weeks of treatment, whereas those infected with HCV genotype 1 (representing about 70%–75% of infected persons in the United States) have a much lower rate of SVR (40%) despite 48 weeks of treatment.<sup>6</sup>

Recently, several highly correlated common single nucleotide polymorphisms (SNPs) on a linkage disequilibrium block in the vicinity of 3 *IFN*- $\lambda$  genes on chromosome 19, encoding interferon  $\lambda 1$  (*IL29*),  $\lambda 2$  (*IL28A*), and  $\lambda 3$  (*IL28B*), have been implicated in response to PEG-IFN/RBV among patients infected with HCV genotype 1 from 3 genome-wide association studies.<sup>7-9</sup> The risk genotype associated with nonresponse to therapy was more

*Abbreviations used in this paper:* IL, interleukin; PEG-IFN/RBV, pegylated interferon and ribavirin; SNP, single nucleotide polymorphism; SVR, sustained virologic response.

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Infection with the hepatitis C virus (HCV) is a global health problem, with worldwide estimates of 120–130 million carriers.<sup>1</sup> In the United States alone, 3–4 million

common in African Americans and thought to account for half of the observed ethnic variation in treatment response. Paradoxically, the responder genotype was associated with higher viral load.

We sought to confirm and extend our understanding of this recently reported genetic association in a cohort of patients with chronic HCV infection from a large tertiary care setting, including subjects infected with HCV genotypes 2 and 3.

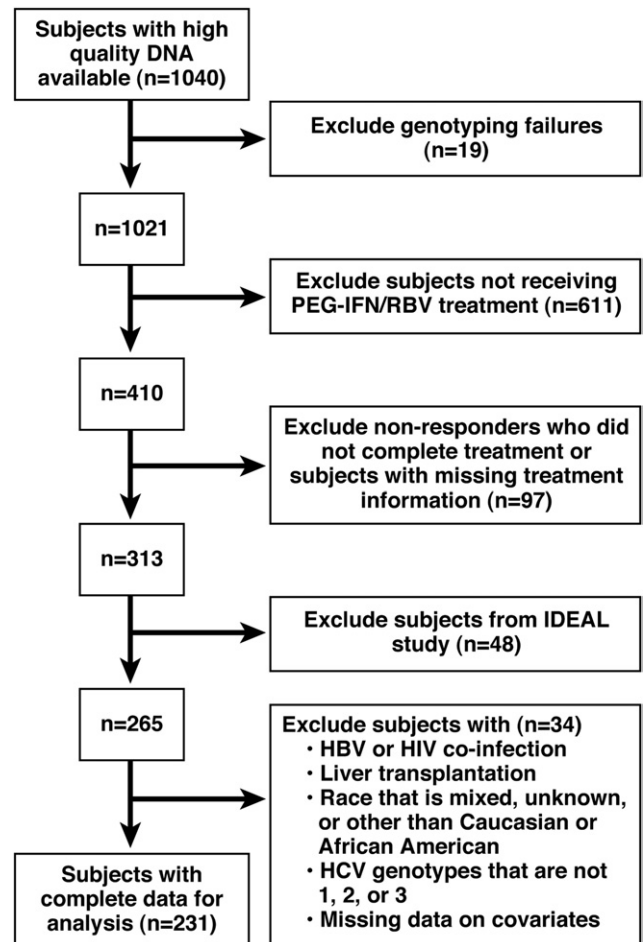
## Materials and Methods

### Patient Population

The Duke Hepatology Clinical Research Database and Repository is an ongoing registry of HCV-infected subjects initiated in 1992, representing a large, well-phenotyped collection of North American chronic HCV patients.<sup>10</sup> All subjects referred to the Duke Liver Clinic with a diagnosis of HCV infection are eligible to be included in this database. Patients are enrolled at the time of their initial clinic visit, and informed consent is obtained for the collection and storage of serum, liver tissue, and peripheral blood for DNA extraction. HCV status is confirmed by the presence of detectable serum HCV RNA. The database includes clinical and demographic data extracted from the medical record, laboratory reports, and case-report forms for those patients enrolled in clinical trials.

The degree of liver fibrosis was determined in liver biopsies scored by a panel of expert histopathologists using the METAVIR scoring system (F0–F4) and coded as a 3-level variable (mild, F0–F1; moderate, F2; severe, F3–F4) for analysis. HCV genotype was determined by the INNOLIPA HCV assay (Innogenetics, Zwijnaarde, Belgium).<sup>11</sup> Treatment history was defined as naive (no previous treatment for HCV infection) vs prior ( $\geq 1$  previous courses of treatment for HCV infection). For the analysis of response to standard-of-care PEG-IFN/RBV, subjects with SVR were defined as having undetectable HCV-RNA levels 24 weeks after cessation of treatment. Those who did not achieve SVR consisted of patients whose HCV-RNA levels remained detectable at the end of treatment (end-of-treatment nonresponders), as well as relapsers, who had undetectable levels of HCV RNA at the end of treatment, but detectable HCV-RNA levels at 24 weeks after cessation of treatment. Race, an important potential confounder of genetic association studies, was self-defined by participants.

For our study, we identified all subjects from the database who had high-quality DNA available for genetic studies ( $n = 1040$ ). Among these, 1021 were genotyped successfully for the rs12979860 polymorphism (98.2% success rate). For analysis of treatment response, we excluded those who were not treated at all as well as those having only received treatment other than PEG-IFN/RBV ( $n = 611$ ). Among patients treated with PEG-IFN/RBV, we included all who attained a SVR, but to restrict our



**Figure 1.** Flow diagram illustrating the selection of subjects from the Duke database for analysis.

analysis to biological nonresponders, we excluded nonresponder subjects who had not received the full course of the per-protocol planned treatment, or in whom treatment response information was not available ( $n = 97$ ). We also excluded 48 patients who also may have been included in the individualized dosing efficacy vs flat dosing to access optimal pegylated interferon therapy (IDEAL) genetic study, which first identified rs12979860 associated with treatment response.<sup>7</sup> Finally, we excluded 34 subjects who were either co-infected with hepatitis B virus or human immunodeficiency virus-1; who had undergone liver transplantation; were of a race that was mixed, unknown, or other than Caucasian or African American; had HCV genotypes that were not 1, 2, or 3; or were missing data on key covariates (Figure 1).

We limited our analysis of viral load to available measurements taken either from treatment-naive subjects or from treated patients before treatment or after completion of treatment for nonresponders. These subjects were drawn from the larger dataset and not restricted to those included in the analysis of treatment response. However, overlapping subjects within the IDEAL study were ex-

cluded. All serum HCV-RNA quantitations were measured using either the National Genetics Institute Super-Quant assay (Culver City, Los Angeles, CA) or the Cobas TaqMan HCV Test (Roche Molecular Systems, Pleasanton, CA) and classified as low (<600000 IU/mL) or high ( $\geq$ 600000 IU/mL) for analysis. Odds ratios were calculated comparing high and low viral load.

This study, the database, and repository were approved by the Duke University Institutional Review Board. All patients provided written informed consent and the study was conducted in accordance with provisions of the Declaration of Helsinki and Good Clinical Practice guidelines.

### Genotyping

The associated interval reported in the 3 genome-wide association studies contains several highly correlated SNPs around the *IL28B* gene. We selected the most strongly associated SNP from the Ge et al<sup>12</sup> study, rs12979860, located upstream of the gene, for genotyping in our cohort using the 5' nuclease assay with allele-specific TaqMan probes. This SNP was associated with treatment response in both Caucasians and African Americans and is in strong linkage disequilibrium (correlated) with the SNPs reported in the other 2 studies.<sup>7</sup> Genotyping was performed in the Duke Institute for Genome Sciences and Policy Genotyping Core and was conducted in a blinded fashion relative to HCV treatment status and other characteristics. Genotyping calls manually were inspected and verified before release. Hardy-Weinberg equilibrium was assessed in Caucasians and African Americans separately.

### Statistical Analysis

SAS statistical software, version 9.1 (SAS Institutes, Cary, NC), was used to perform logistic regression analysis of rs12979860 genotypes associated with treat-

ment response, and subsequently viral load. Rs12979860 genotypes were coded to test both an additive model, defined as 0, 1, or 2 copies of the C allele, and a recessive model, comparing subjects homozygous (CC) with those with one or no copies (CT/TT) of the C allele. Homogeneity of genotype effects across strata was tested by introducing an interaction term. Analysis of response to PEG-IFN/RBV was performed in subjects with valid treatment response data, whereas analysis of rs12979860 associations with HCV genotype and with viral load was performed in a larger number of patients from the extended cohort. Primary analysis of the association between rs12979860 and treatment response controlled for age, sex, HCV genotype, fibrosis score, and treatment history and was stratified by race. To assess the relative contribution of key established predictors of treatment response, a multivariable model was run in the combined population of African Americans and Caucasians controlling for race and the same covariates described earlier, as well as viral load (available on a subset of the population). Likelihood ratios were calculated as follows: sensitivity/(1 - specificity) for LR+ and (1 - sensitivity)/specificity for LR-. Overall model fit was assessed using the Hosmer-Lemeshow-Cressie goodness-of-fit statistic,<sup>13</sup> the C statistic, and Nagelkerke R<sup>2</sup><sup>14</sup> (both bias-corrected) using the Design library of the R statistical language.<sup>15-16</sup> To assess the value of rs12979860 in reclassifying treatment response we computed the Net Reclassification Improvement,<sup>17</sup> which quantifies reclassification of the outcome when adding a predictor to the model. Multinomial regression was used to model the association of rs12979860 with treatment response as a 3-level variable: SVR, relapser, and end-of-treatment nonresponse. Association between rs12979860 genotype and viral load was performed in a logistic regression model controlling for age, sex, HCV genotype, and fibrosis.

**Table 1.** Characteristics of Chronic HCV Patients Undergoing Treatment With PEG-IFN/RBV by Overall Treatment Response

Variable	All (N = 231)	Non-SVR (N = 159)	SVR (N = 72)	P
Mean age $\pm$ standard deviation, y	47.7 $\pm$ 6.8	48.3 $\pm$ 6.6	46.4 $\pm$ 7.1	.0598
Sex, male (%)	150 (64.9)	106 (66.7)	44 (61.1)	.4129
Race (%)				.0004
Caucasian	178 (77.1)	111 (69.8)	67 (93.1)	
African American	53 (22.9)	48 (30.2)	5 (6.9)	
METAVIR fibrosis stage (%)				.4331
F0-F1	42 (18.2)	27 (17.0)	15 (20.8)	
F2	88 (38.1)	58 (36.5)	30 (41.7)	
F3-F4	101 (43.7)	74 (46.5)	27 (37.5)	
HCV genotype (%)				$1.01 \times 10^{-7}$
1	186 (80.5)	144 (90.6)	42 (58.3)	
2/3	45 (19.5)	15 (9.4)	30 (41.7)	
Treatment history (%)				$6.06 \times 10^{-5}$
Naive	147 (63.6)	87 (54.7)	60 (83.3)	
Prior	84 (36.4)	72 (45.3)	12 (16.7)	
Baseline viral load (%), N = 151				.1533
<600000 IU/mL	55 (36.4)	29 (31.9)	26 (43.3)	
$\geq$ 600000 IU/mL	96 (63.6)	62 (68.1)	34 (56.7)	

## Results

### Association of rs12979860 Genotype With SVR to PEG-IFN/RBV by Race

A description of the cohort used in our analysis of response to PEG-IFN/RBV is shown in Table 1. Patients predominantly were Caucasian, infected with HCV genotype 1, and broadly representative of a tertiary care chronic HCV cohort. Among patients treated with PEG-IFN/RBV, those included in this analysis differed only slightly from those excluded, with more HCV genotype 2/3-infected subjects (20% vs 11%;  $P = .02$ ). As expected, responders to standard-of-care PEG-IFN/RBV treatment were characterized as having significantly lower viral load, being younger, of Caucasian race, infected with HCV genotype 2 or 3, and being treatment-naive. Rs12979860 allele frequencies differed by race and were consistent with those previously reported (Caucasian C, 0.63; T, 0.37; African American C, .40; T, .60). In the overall cohort, a nominal deviation in Hardy-Weinberg equilibrium was found for Caucasians ( $P = .02$ ), which could not be

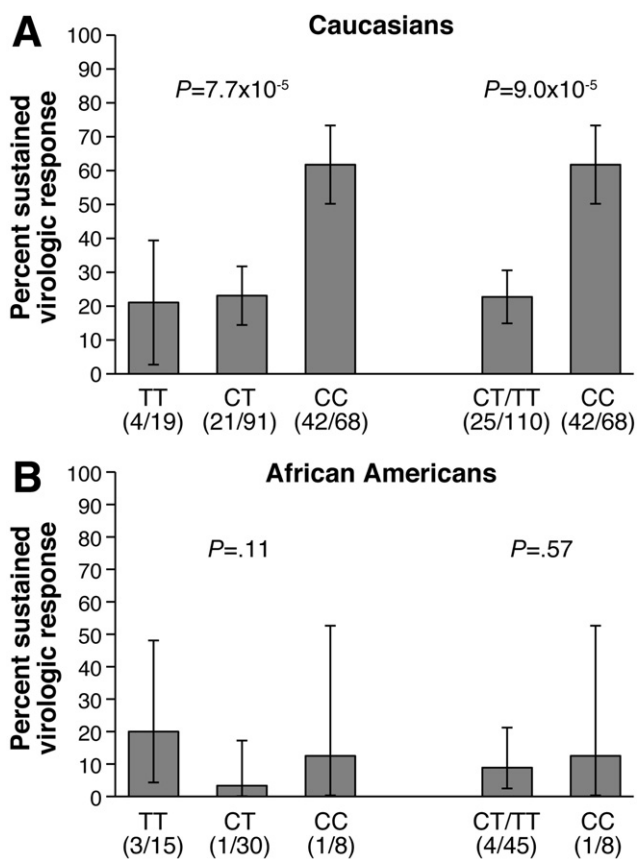
attributed to genotyping error, but possibly reflects an underlying association between genotype and chronic HCV infection.

Figure 2 illustrates how, among Caucasians, rs12979860 genotype was associated significantly with response to PEG-IFN/RBV, with the greatest effect seen when comparing subjects with two copies of the C allele (CC genotype) with those with one or no copies. Therefore, all subsequent analyses considered the CC genotype vs CT/TT. This association remained significant in multivariable analysis controlling for age, sex, HCV genotype, treatment history, and fibrosis, where presence of the CC genotype conferred a nearly 6-fold increased odds of SVR relative to the CT/TT genotype (odds ratio, 5.79; 95% confidence interval, 2.67–12.57;  $P = 9.0 \times 10^{-6}$ ). Among Caucasians, there was no difference in the effect of rs12979860 on SVR comparing subjects with HCV genotype 1 and 2/3, or between those with a prior treatment history and those treatment naive at baseline (rs12979860 genotype by trait interactions,  $P > .05$ ).

African Americans comprised only 23% of our cohort and predominantly were infected with genotype 1 HCV (98%), and 57% were treatment-naive. As illustrated in Figure 2, no association was found between rs12979860 genotype and treatment response in African Americans in our study, and the difference in the rs12979860 treatment response association between African Americans and Caucasians did not reach significance (rs12979860 by race interaction,  $P = .34$ ). However, it should be noted that the estimated proportion of SVR among patients with the CC genotype, 0.125 (1 of 8), is unstable because of the small sample size, resulting in a wide confidence interval (0.003–0.53), and thus making it difficult to draw inference.

To assess the relative contribution of important covariates to treatment response, we examined the effect of each covariate in a multivariable model. Table 2 shows the relative effect of all covariates on treatment response in univariable and multivariable models. HCV genotype was the strongest predictor of treatment response in univariable and multivariable analyses that did not include the rs12979860 genotype. Inclusion of rs12979860 in the model attenuated the effects of HCV genotype and enhanced the association with viral load. Rs12979860 genotype emerged as the strongest factor associated with treatment response; other independently associated factors included HCV genotype, viral load, age, and treatment history. Inclusion of rs12979860 genotype in the model improved the C statistic (from 0.75 to 0.82) as well as the Nagelkerke  $R^2$  (from 0.25 to 0.38). In the current study, inclusion of the IL28B genotype in the model resulted in reclassification of responders (30%) and nonresponders (56%), for an overall Net Reclassification Improvement (0.86) that was highly significant ( $P = 2.3 \times 10^{-7}$ ).

To assess the relative value of rs12979860 compared with HCV genotype, we calculated sensitivity, specificity,



**Figure 2.** Association between rs12979860 genotype and PEG-IFN/RBV treatment response in Caucasian and African American chronic HCV patients from our cohort. (A) Caucasians, (B) African Americans. Numbers with SVR out of all with that genotype are shown below each rs12979860 genotype. Chi-squared  $P$  values for Caucasians are adjusted for age, sex, HCV genotype, treatment history, and fibrosis.  $P$  values in African Americans are from the Fisher exact test and are not adjusted because of the small sample sizes.

**Table 2.** Odds Ratios Associated With Predictors of SVR to PEG-IFN/RBV Among 151 Chronic HCV Patients With Complete Data, Including Viral Load

Predictor	Unadjusted OR (95% CI)	P	Adjusted OR <sup>a</sup> (95% CI)	P	Adjusted OR <sup>b</sup> (95% CI)	P
rs12979860 CC	—	—	—	—	7.88 (3.10–20.05)	1.48 × 10 <sup>-5</sup>
Age (per year)	0.94 (0.89–1.00)	.0316	0.94 (0.88–1.00)	.0385	0.92 (0.86–1.00)	.0387
Female sex	1.42 (0.70–2.87)	.3263	1.62 (0.71–3.69)	.2556	1.91 (0.76–4.80)	.1720
Caucasian race	4.73 (1.55–14.50)	.0065	2.61 (0.75–9.09)	.1323	1.61 (0.41–6.25)	.4921
HCV genotype 2/3	6.97 (2.96–16.42)	9.01 × 10 <sup>-6</sup>	8.35 (3.10–22.53)	2.78 × 10 <sup>-5</sup>	7.20 (2.47–20.98)	.0003
Fibrosis, mild	1.21 (0.49–3.01)	.9359	0.80 (0.26–2.46)	.6930	0.97 (0.29–3.27)	.9586
Fibrosis, moderate	1.57 (0.76–3.24)	.3155	1.93 (0.81–4.60)	.1366	2.08 (0.81–5.37)	.1302
Treatment-naive	2.39 (1.11–5.11)	.0253	3.47 (1.37–8.82)	.0090	3.23 (1.15–9.09)	.0260
Low viral load	1.64 (0.83–3.21)	.1533	2.10 (0.94–4.69)	.0715	3.58 (1.41–9.10)	.0073

NOTE. The unadjusted odds ratio (Unadj. OR) and 95% confidence interval (CI) are presented for each covariate.

<sup>a</sup>The adjusted odds ratio included age, sex, race, HCV genotype, fibrosis, treatment history, and viral load in the model. Odds ratios are for rs12979860 (CC vs CT/TT); age (per year); sex (female vs male); race (Caucasian vs African American); HCV genotype (2/3 vs 1); fibrosis (mild vs severe and moderate vs severe); treatment history (naive vs prior); and viral load (low vs high).

<sup>b</sup>The adjusted odds ratio also included rs12979860 genotype. Odds ratios are for rs12979860 (CC vs CT/TT); age (per year); sex (female vs male); race (Caucasian vs African American); HCV genotype (2/3 vs 1); fibrosis (mild vs severe and moderate vs severe); treatment history (naive vs prior); and viral load (low vs high).

and likelihood ratios in our cohort of Caucasian subjects (Table 3). Comparing rs12979860 CC genotype with HCV genotype 2/3 revealed HCV genotype as having somewhat better specificity, but rs12979860 genotype has better sensitivity and is more common, found in 38% of chronic HCV patients (compared with ~25% prevalence of HCV genotypes 2/3). Performance of rs12979860 among the subset of HCV genotype 1 subjects, or among the subset of treatment-naive subjects, mirrors that in all subjects with HCV genotypes 1, 2, and 3 combined.

**Effect of rs12979860 on Treatment Relapse Among Caucasians**

Among 111 (62%) Caucasian subjects who failed to achieve SVR to PEG-IFN/RBV, 29 (26%) were classified as relapsers. The frequency of the rs12979860 CC genotype in this group was between that observed for the SVR and end-of-treatment nonresponder groups. However, only the difference found between relapsers and end-of-treatment nonresponders was significant, suggesting that rs12979860 CC genotype influences end-of-treatment response and does not distinguish accurately between patients with a relapse response and those with a SVR (Figure 3).

**Association With HCV Genotype**

HCV genotype data were available for a larger group of patients than were used for analysis of treat-

ment response (n = 649 Caucasians). We compared the rs12979860 genotype frequencies between Caucasians infected with HCV genotypes 1, 2, and 3 (Figure 4). Rs12979860 CC was most common in genotype 3 patients (55%), followed by genotype 2 (46%) and genotype 1 (33.5%; P = .0007). To correct for possible referral bias, we examined this association in the subset of naive patients undergoing treatment with PEG-IFN/RBV and observed the same trend (data not shown).

**Association With Viral Load**

Off-treatment viral load measures were available for 301 Caucasians with chronic HCV infection from our cohort, 277 of whom had complete data on covariates as well and could be used in our analysis to determine the association between rs12979860 genotype and viral load. The odds of having a higher quantitative viral load in patients with rs12979860 CC genotype were twice that of patients with genotype CT/TT in analysis controlling for age, sex, HCV genotype, and fibrosis (odds ratio, 2.13; 95% confidence interval, 1.24–3.66; P = .0061).

**Discussion**

Treatment decisions in patients with chronic hepatitis C infection currently are based on clinical, demographic, and virologic characteristics of infected patients. Although these may be helpful from a population point

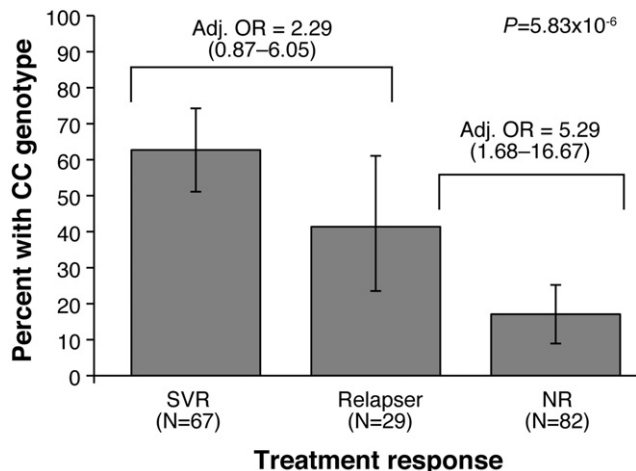
**Table 3.** Comparison of Performance of HCV Genotype and rs12979860 CC Genotype for Predicting SVR With PEG-IFN/RBV Among the 178 Chronic HCV Caucasian Patients

Model <sup>a</sup>	HCV genotype	Treatment history	Predictor	% with predictor	Sensitivity	Specificity	LR+	LR–
178	1, 2, 3	All	HCV genotype 2/3	25	45%	87%	3.46	0.63
178	1, 2, 3	All	Rs12979860 genotype CC	38	63%	77%	2.74	0.48
117	1, 2, 3	Naive	Rs12979860 genotype CC	42	64%	77%	2.78	0.47
134	1	All	Rs12979860 genotype CC	34	65%	78%	2.95	0.45

LR+, likelihood ratio positive; LR–, likelihood ratio negative.

<sup>a</sup>The number of subjects used in the model to calculate the test parameters.

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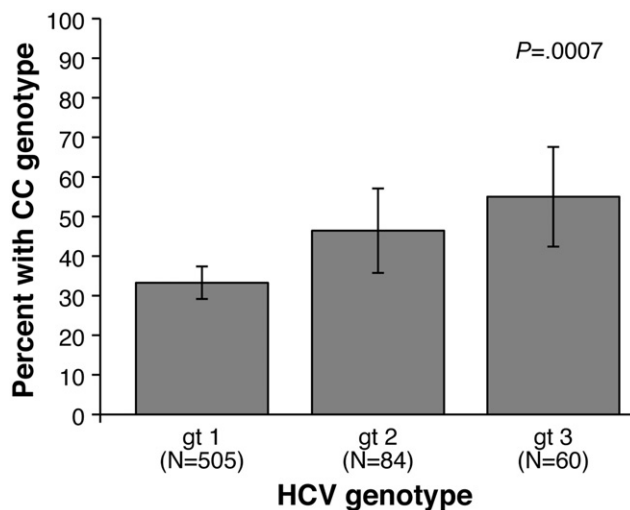
**Figure 3.** Effect of rs12979860 genotype on 3-level treatment outcome in Caucasian chronic HCV patients treated with PEG-IFN/RBV. Adjusted odds ratios (ADJ. OR) and 95% confidence intervals are for rs12979860 CC vs CT/TT genotype comparing SVRs with relapsers, and relapsers with end-of-treatment nonresponders (NR), while controlling for age, sex, HCV genotype, treatment history, and fibrosis score. The *P* value shown is for the effect of the rs12979860 CC genotype on 3-level treatment response in multinomial regression analysis.

of view, for any individual patient and provider, these baseline pretreatment characteristics are inaccurate in predicting treatment response in hepatitis C patients infected with genotype 1, the most common genotype found in the United States. We have confirmed in this study that the recently identified common genetic variant in the IFN- $\lambda$  gene region is associated strongly with response to standard-of-care PEG-IFN/RBV in a tertiary care setting. The association among Caucasians thus was validated outside of the clinical trial setting. Because of the limited sample size of African Americans and other racial groups in this current study, we had inadequate power to detect statistical association in these groups, as was observed by Ge et al.<sup>7</sup> Nonetheless, inclusion of rs12979860 genotype and race in a multivariable model caused an attenuation of the effect of race on treatment response, indicating that the effect of race may be mediated in part through this polymorphism.

Our results extend the observations of Ge et al<sup>7</sup> and indicate that, among Caucasians, rs12979860 has a high specificity, similar to HCV genotype, the current best baseline indicator used for identifying patients who will achieve a SVR to antiviral therapy. Used in conjunction with HCV genotype, rs12979860 may provide additional discriminatory power to identify likely responders to treatment.

Another interesting observation was the significantly different frequency of the rs12979860 genotype according to HCV genotype in Caucasian patients. HCV genotype 1-infected patients were less likely to have the rs12979860 response genotype than were patients infected with HCV genotypes 2 or 3. Although interesting,

this relationship does not fully explain why patients infected with HCV genotypes 2 or 3 have significantly higher sustained response rates compared with those infected with HCV genotype 1; both the gene variant and HCV genotype remain independently associated with treatment response in the multivariable models we ran. Nonetheless, this observation theoretically could have some biological bearing on the development of persistent or chronic (vs spontaneously cleared) HCV infection. The data could be interpreted in the context of a case-only study design, where an association between 2 factors (rs12979860 and HCV genotype) among cases of chronic HCV may reflect interaction between rs12979860 and HCV genotype in the development of chronic HCV.<sup>18,19</sup> Based on these results, we would hypothesize that subjects with both HCV genotype 2/3 and rs12979860 CC would be more likely to develop chronic HCV and hence less likely to clear the virus spontaneously than those with HCV genotype 1 and rs12979860 CT/TT. Although this relationship may seem counterintuitive on account of HCV genotype 2/3 subjects having a significantly higher rate of treatment-induced viral clearance, it warrants further exploration in appropriate prospective studies of spontaneous clearance. We also considered that the association could have been due to referral bias, whereby chronic HCV patients who are difficult to treat may be overrepresented among the Duke cohort, and these patients would be more likely to be HCV genotype 1 and rs12979860 CT/TT genotype. This bias could lead to an overestimate of the association between rs12979860 genotype and HCV genotype. However, when we analyzed a subset of treatment-eligible patients with no prior treatment history we found a similar trend of association.



**Figure 4.** Association between rs12979860 genotype and HCV genotype among 681 Caucasian chronic HCV patients from our cohort. Percentage of subjects carrying the rs12979860 CC genotype is shown with 95% confidence intervals. Chi-squared *P* value for association between HCV genotype (gt1, gt2, gt3) and rs12979860 CC vs CT/TT genotype is shown.

Although the rs12979860 SNP showed a remarkably strong association with treatment response in both the study by Ge et al<sup>7</sup> and our replication study, the exact causal variant underlying the observed genetic association has not been identified. This SNP lies closest to 2 genes encoding for IFN- $\lambda$  proteins,  $\lambda 2$  and  $\lambda 3$ . IFN- $\lambda$  have been studied previously in the context of HCV infection and shown to suppress HCV replication in vitro.<sup>20–22</sup> It is possible that the causal variant affects expression or function of one of these antiviral genes, which may in turn affect viral control.

Interestingly, Ge et al<sup>7</sup> reported a paradoxical relationship to viral load in that the responder allele of rs12979860 was associated statistically with a higher baseline viral load, inconsistent with the clinical observation that higher baseline viral load typically is associated with a poorer treatment response.<sup>4–6</sup> We also confirmed this paradoxical association with off-treatment viral load in our expanded cohort of Caucasian patients from a tertiary care setting. The exact mechanism underlying this genetic association with high viral load and yet increased likelihood of treatment response remains to be determined.

There are several important clinical implications of this genetic association originally reported by Ge et al<sup>7</sup> in a clinical trial population and confirmed in the present study in a tertiary care population. First, the rs12979860 SNP may be useful in determining which chronic HCV genotype 1 patients would be most likely to respond to treatment with PEG-IFN/RBV. Given the high prevalence of the rs12979860 responder genotype in Caucasians (~40%), this could have a substantial impact on treatment decision making. Our data support the potential utility of rs12979860 in predicting SVR in Caucasians, regardless of treatment history. However, additional prospective studies are needed to determine the true predictive value of this marker among all treatment-eligible patients, including those from other racial/ethnic and HCV genotype groups, and taking into account noncompliant and relapsing patients as well. Ultimately, chronic HCV patients with the rs12979860 responder genotype may be more motivated to comply with treatment, or to undergo treatment in the presence of mild underlying liver disease, knowing that they have a higher likelihood of SVR.

These important genetic findings related to treatment response in patients with chronic hepatitis C infection suggest the possibility of personalized medicine for the treatment of this disease. Clinical trials are now necessary to determine whether HCV genotype 1-infected patients with the favorable rs12979860 responder genotype would benefit equally from shorter treatment duration with our current therapies or future therapies, thus reducing the cost and side effects associated with longer-term treatment. How the rs12979860 responder genotype influences the outcomes to future antiviral strategies, including those based on protease and polymerase inhibition,

requires immediate attention and investigation. Understanding the clinical implications of this genetic, biologically plausible finding will be a major research agenda and priority.

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#### *Conflicts of interest*

These authors disclose the following: Drs Thompson and McHutchison are co-inventors with Schering Plough on a patent application on the original finding of rs12979860 association with pegylated interferon and ribavirin treatment response in HCV infection; Drs McHutchison and Muir have received research funding from and acted in an advisory capacity for Schering Plough. The remaining authors disclose no conflicts.

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