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Quantitation of Pretreatment Serum IP-10 Improves the Predictive Value of an *IL28B* Gene Polymorphism for Hepatitis C Treatment Response

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Abstract

Polymorphisms of *IL28B* gene are highly associated with sustained virological response (SVR) in patients with chronic hepatitis C treated with peginterferon and ribavirin. Quantitation of Interferon-γ Inducible Protein-10 (IP-10) may also differentiate antiviral response. We evaluated IP-10 levels in pretreatment serum from 115 non-responders and 157 sustained responders in the VIRAHEP-C cohort, including African Americans (AA) and Caucasian Americans (CA). Mean IP-10 was lower in sustained responders compared to non-responders (460 ± 37 pg/ml vs 697 ± 49 pg/ml, $p<0.001$), both in AA and CA. The positive predictive value of low IP-10 levels (<600 pg/ml) for SVR was 69% while the negative predictive value of high IP-10 levels (>600 pg/ml) was 67%. We assessed the combination of pretreatment IP-10 levels with *IL28B* genotype as predictors of treatment response. The *IL28B* polymorphism rs12979860 was tested in 210 participants. CC, CT, or TT genotypes were found in 30%, 49%, and 21%, respectively, with corresponding SVR rates of 87%, 50%, and 39% ($p<0.0001$). Serum IP-10 levels within the *IL28B* genotype groups provided additional information regarding the likelihood of SVR ($p< 0.0001$). CT carriers with low IP-10 had 64% SVR versus 24% with high IP-10. Similarly, a higher SVR rate was identified for TT and CC carriers with low versus high IP-10 (TT: 48% versus 20%, CC: 89% versus 79%). *IL28B* genotype and baseline IP-10 levels were additive but independent when predicting SVR in both AA and CA.

Conclusions—When *IL28B* genotype is combined with pretreatment serum IP-10 measurement, the predictive value for discrimination between SVR and non-response is significantly improved, especially in non-CC genotypes. This relationship warrants further investigation to elucidate mechanisms of antiviral response and prospective validation.

Keywords

Peginterferon; Ribavirin; Race; Biomarker; Genotype

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Hepatitis C (HCV) is a single stranded RNA virus that usually establishes persistent infection in its host. Of patients exposed to HCV, about 80% will develop chronic viral infection characterized by liver infiltration of HCV-specific and nonspecific T cells accompanied by “proinflammatory” cytokines resulting in damage to virus-infected, as well as bystander, hepatocytes with resultant fibrosis formation. About 30–35% will develop cirrhosis and once a patient is cirrhotic, there is a 1–4% annual rate of hepatocellular carcinoma development (1).

Combined treatment with peginterferon (pegIFN) and ribavirin achieves sustained virological response (SVR) in 42–52% of genotype 1 patients (2–4). Unfortunately, the remainder either fail to respond, or must discontinue treatment prematurely due to adverse events. Response rates to pegIFN and ribavirin are associated with both viral and host factors. Pretreatment predictors of non-response include genotype 1 infection, high viral load (>800,000 IU/mL), advanced fibrosis or cirrhosis, high body mass index (BMI), age older than 40, and African American race (2–4). Currently, on-treatment predictors of response to pegIFN and ribavirin include viral kinetics at weeks 4 and 12. Patients who do not attain an early virologic response have only a 1–3% chance of viral clearance and therapy is usually halted (2, 5). Conversely, 87% of those who achieve a rapid virological response (defined as HCV RNA undetectable at week 4 of therapy) achieve SVR (6). While viral kinetics have proven useful, better predictors of SVR and non-response would be helpful to identify patients with the best chance of response before the initiation of combination antiviral therapy.

The US population has proven to be a more difficult group to treat than many others with lower SVR rates, perhaps due in part to higher BMI’s and a greater racial variation. African-Americans (AA) harbor predominantly genotype 1 virus and have notably lower overall response rates to pegIFN and ribavirin (approximately 26–28%) compared to Caucasian Americans (CA) (7–9). Determining why AA patients respond less well to antiviral therapy with pegIFN and ribavirin compared to CA was the focus of The Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C (VIRAHEP-C). This NIH-sponsored study examined a variety of clinical, immunologic, virologic, and host genetic causes for the lack of response to treatment, although no single factor was identified that could account for the diminished rate of response (8, 10–12).

Chemokines and cytokines are attractive as potential markers for treatment outcome as they are regulators of immunity and inflammation in HCV infection. Many are modulated by exogenous interferon and play critical roles in viral clearance. Responders tend to have a lower baseline activation of the immune system prior to treatment that is more markedly induced in response to IFN treatment (13–15). Several studies have shown that the non-ELR CXC chemokine interferon-γ (IFNγ) inducible protein-10 (IP-10 or CXCL10) may be a prognostic marker for HCV treatment outcome in genotype 1 infection (15–20). Elevated pretreatment IP-10 levels correlate with nonresponse to pegIFN and ribavirin therapy although this relationship has not been validated in AA patients.

More recently, data has been published on a gene polymorphism (rs12979860) upstream of *IL28B* that is favorably associated with treatment response to pegIFN and ribavirin in both AA and Caucasian patients (21). Regardless of race, carriage of the C allele increases treatment response rates with those who have the CC genotype having the highest SVR rates, CT intermediate and TT the lowest (21). This favorable genotype is seen more frequently in Caucasian patients and likely explains about half of the difference in response between AA and CA of European ancestry. The *IL28B* gene encodes interferon-λ-3 which is a type III IFN induced by viral infections (22, 23). While the mechanism underlying the

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association of *IL28B* genotype and HCV clearance has not been elucidated, modulation of the innate immune response is likely playing a role in control of this viral infection. *IL28B* genotyping may provide useful pretreatment stratification of patients for HCV treatment in the future but it does not completely explain response discrepancies between AA and CA patients.

In this study, we measured pre-treatment IP-10 levels in serum samples from 272 patients in the VIRAHEP-C cohort (115 non-responders and 157 SVR). This analysis demonstrated IP-10 to be equally predictive of SVR in both CA and AA patients. We then assessed the combination of pretreatment serum IP-10 levels with *IL28B* genotype as predictors of response to pegIFN and ribavirin in this cohort.

PATIENTS AND METHODS

Patients

The VIRAHEP-C study was a multicenter study of combination pegIFN and ribavirin therapy of chronic hepatitis C designed to assess the rates and predictors of response among AA and CA with genotype 1 infection, and to identify reasons for nonresponse to therapy. The design and primary outcomes of the VIRAHEP-C trial have been reported elsewhere (8). Adults who were treatment naive, infected with genotype 1, had detectable HCV RNA, and had histologic evidence of chronic HCV were eligible to participate. Patients were classified by race as either African American or Caucasian, and by ethnicity as either Hispanic or non-Hispanic, based on self-report. All participants were required to have been born in the United States. From 8 clinical centers across the United States, 401 patients were enrolled and started on therapy between July 2002 and December 2003.

For the present study, serum samples were acquired from a subset of 272 patients from the total VIRAHEP-C cohort, comprising 157 sustained virological responders (SVR) (104 CA, 53 AA) and 115 non-responders (34 CA, 81 AA). All specimens analyzed in this study were obtained under IRB-approved protocols for which participants provided written informed consent, including consent for genetic testing.

Treatment

Patients received peginterferon alfa-2a (Pegasys, Roche Pharmaceuticals, Nutley, NJ) 180ug weekly and ribavirin (Copegus, Roche Pharmaceuticals, Nutley, NJ) 1000–1200 mg daily for at least 24 weeks. Patients who became HCV RNA negative by week 24 continued treatment for a total of 48 weeks, whereas those who remained HCV RNA positive stopped treatment and were considered non-responders. The primary endpoint of the trial was SVR, defined as the absence of detectable HCV RNA for at least 24 weeks after stopping therapy.

HCV RNA quantification

HCV RNA testing was done at a central laboratory (SeraCare BioServices, Gaithersburg, MD) using the Cobas Amplicor Assay (sensitivity 50 IU/ml: Roche Molecular Diagnostics, Alameda, CA). Selected samples were tested for HCV RNA levels by Cobas Amplicor Monitor Assay and for HCV RNA genotype by Versant HCV Genotype Assay (Bayer, Tarrytown, NY).

Liver histology

All patients had undergone liver biopsy within 18 months of screening, which were read blinded by a central pathologist. All biopsies were assessed for severity of hepatitis C by grading the inflammation and staging the fibrosis using Ishak's modified histologic activity index (HAI) scoring system.

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Quantification of serum IP-10

Interferon-γ Inducible Protein-10 (IP-10) was measured in serum samples collected at baseline, prior to initiation of treatment, using the commercially available Quantikine human CXCL10/IP-10 immunoassay (R&D Systems). All samples were diluted 1:2 and analyzed in duplicate. The linear dynamic range of the IP-10 measurement in this assay was 8 – 500 pg/ml, with a detection limit at 7.8 pg/ml. Samples with IP-10 concentration above 1000 pg/ml were diluted 1:5 and re-analyzed.

Genotyping of *IL28B*

The *IL28B* polymorphic marker rs1297860 was analyzed using the ABI TaqMan allelic discrimination kit and the ABI7900HT Sequence Detection System (Applied Biosystems), as described by Thomas et al (21, 24–27). DNA samples were genotyped from 210 patients from our cohort.

Statistical methods

Standard statistical analyses were done using JMP 7.0.2 or SAS version 9.1 software (both from SAS Institute, Inc). IP-10 concentrations were log-transformed before use in statistical tests to meet distribution normality assumptions. Publicly available packages in R (version 2.8.0) were used to assess different classification models (diagonal linear discriminant analysis, random forest, support vector machine, and bagging), as well as receiver operating characteristic (ROC) curve analysis. Fitting logistic regression models and generalized linear models was performed using respectively the *proc logistic* and *proc genmod* procedures in SAS. Graphs were made with the utilized statistical software tools or with GraphPad Prism 4 (GraphPad Software, Inc).

RESULTS

Patients included in the study cohort

Serum samples from 157 sustained virologic responders (SVR) and 115 non-responders to antiviral therapy were included from the VIRAHEP-C cohort for this study. The definitions of SVR and non-responder are provided in the methods section. Patients with viral relapse, breakthrough or less than 12 weeks of available virologic data were excluded. The cohort consisted of 134 AA and 138 CA. Baseline patient characteristics of this cohort were as follows: age 48.4 ± 7.4 years; viral load 4.6 ± 5.7 $\times 10^6$ IU/ml; platelet count 214 ± 73 $\times 10^6$ cells/mm^3; alanine transaminase (ALT) 90.9 ± 72.9 IU/l; total bilirubin 0.70 ± 0.35 mg/dl; albumin 4.1 ± 0.40 g/dl; and hematocrit 43.2 ± 3.8 % (all data as mean ± SD, Supplementary Table 1). The cohort included 96 females and 176 males, and 19% with an Ishak fibrosis score of 4–6. Samples from 210 of the 272 patients in our cohort were available for *IL28B* genotyping, 123 SVR and 87 non-responders of whom 111 were CA and 99 AA.

Baseline serum IP-10 measurement and treatment response

Mean serum IP-10 levels were significantly lower in SVR compared to non-responder patients (437 ± 31 pg/ml versus 704 ± 44 pg/ml, $p<0.001$) (Figure 1A, Table 1). To assess the potential predictive value of IP-10 measurements, we stratified the patients according to a 600 pg/ml threshold value which has been used in prior studies (15, 16, 18). Sixty-nine percent (129/188) of individuals with a low baseline IP-10 level (<600 pg/ml) were responders (positive predictive value, PPV = 69%), while 67% (56/83) of the individuals with a high baseline IP-10 level (>600 pg/ml) were non-responders to therapy (negative predictive value, NPV = 67%) (Figure 1B). Overall, this results in a specificity of 82% (129/157) and a sensitivity of 49% (56/115) for a test predictive of therapy response based on pre-treatment serum IP-10 levels. Baseline demographic parameters of the cohort

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stratified according to pretreatment IP-10 level are shown in Supplementary Table 1. Between high and low IP-10 groups, significant differences were seen for several parameters implying a possible association with IP-10 level. Previous groups have also noted association of race and viral load with IP-10 levels (16, 20).

We evaluated the IP-10 threshold value of 600 pg/ml used to identify SVR patients by computing the cutoff value with the best discriminatory ability based on a ROC curve analysis. In our dataset, a threshold concentration of 370 pg/ml revealed the optimal combination of specificity (80%) and sensitivity (56%) in predicting SVR patients. We then determined our optimal IP-10 level to correctly predict both SVR as well as non-response. A threshold value of 550 pg/ml yielded the highest rate of true positives or negatives (69%), and correlated well with the 600 pg/ml cutoff that has been used in the literature (68% true positives or negatives predicted in our dataset). Finally, logistic regression analysis of pre-treatment IP-10 concentrations enabled fitting the probability of SVR for specific IP-10 levels measured in individual patients, and demonstrated a highly significant effect of IP-10 ($p<0.0001$; Supplementary Figure 1, grey curve).

Effect of race on serum IP-10 levels

When comparing pre-treatment IP-10 serum levels of CA and AA, no significant differences were observed in separate analyses of the responders ($p=0.75$) and non-responders ($p=0.97$) (Table 1). The significant ($p=0.015$) difference in baseline serum IP-10 level between CA and AA that was observed in the overall study cohort can most likely be explained by the unbalanced composition of the cohort (IFN treatment response rate in the CA subgroup was 75% as compared to 40% in the AA subgroup). The highly significant difference in IP-10 serum level between responders and non-responders to IFN therapy was found in both CA and AA (Table 1). Logistic regression analyses of baseline IP-10 levels were used to generate treatment response curves for CA and AA patients (Supplementary Figure 1). The response curves for AA and CA patients revealed a significant effect of both IP-10 ($p<0.0001$) and race ($p<0.0001$), but no significant interaction between IP-10 and race ($p=0.08$).

IL28B genotype and treatment response

Of the 210 patients genotyped, 30% were CC, 49% were CT, and 21% were TT. A significant association between *IL28B* genotype and treatment response was observed: corresponding SVR rates were 87% for CC, 50% for CT, and 39% for TT ($p<0.0001$) (Table 2). For Caucasian-Americans, 49% were CC with an SVR of 91%, 41% were CT with an SVR of 67% and 10% were TT with an SVR of 45% ($p<0.001$). For African-Americans, only 9% were CC with an SVR of 67%, 58% were CT with an SVR of 35% and 33% were TT with an SVR of 36% ($p=0.20$).

Association of *IL28B* genotype and pretreatment IP-10 levels with treatment response

Mean serum IP-10 levels were similar for all patients regardless of *IL28B* genotype both in Caucasians ($p=0.27$) and in African-Americans ($p=0.58$) (Figure 2). This lack of correlation between serum IP-10 and *IL28B* genotype indicates that the associations with SVR observed for both of these markers are independent. Using the 600 pg/ml cutoff for pretreatment IP-10 levels, the SVR rate for our cohort of patients with both serum IP-10 and *IL28B* genotype data available ($n=210$) was 69% for those with a low IP-10 level (<600 pg/ml) and 35% for those with a high IP-10 level (>600pg/ml) ($p<0.0001$).

Modeling SVR as a function of *IL28B* genotype and serum IP-10 (above or below 600 pg/ml) in a nominal logistic regression revealed a significant additive effect of *IL28B* genotype ($p<0.0001$) and serum IP-10 ($p<0.0015$) in predicting SVR ($Chi^2=55$, $p<0.001$), but no

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interaction between *IL28B* and IP-10 (p=0.66). Figure 3 visualizes that baseline IP-10 levels within the *IL28B* genotype groups provided additional and independent information regarding SVR rate. More specifically, baseline IP-10 levels were most helpful in *IL28B* T-allele carriers. The overall response rate for CT carriers was 50% but for those with low IP-10 levels 64% had an SVR versus 24% with high IP-10 levels. For the TT genotype, 39% had an SVR with 48% in the low pretreatment IP-10 group and 20% in the high IP-10 group. Logistic regression modeling of SVR response based on serum IP-10 level treated as a continuous variable and *IL28B* genotype enabled a more individualized prediction of the probability of SVR according to serum IP-10 level, with an additional and significant *IL28B* genotype-dependent shift in response curve (Supplementary Figure 2). Complementary receiver operating characteristic (ROC) curve analyses, which allow a more quantitative comparison of predictive models, revealed similar ROC area under the curve (AUC) values for the model based on pretreatment serum IP-10 alone (0.71) versus *IL28B* genotype alone (0.70). A much higher ROC AUC value (0.80) was achieved, however, for the model that combined both markers (Figure 4). Together, these data demonstrate that combining *IL28B* genotype with pretreatment serum IP-10 measurements clearly improves the predictive value of SVR, especially in non-CC genotypes.

The same and significant trend was also found when the analysis was done per racial group (Table 3). For example, in African Americans, the difference with baseline IP-10 levels was even more striking for the CT and TT *IL28B* genotypes. For the CT carriers with low IP-10, SVR was 48% versus 17% with high IP-10, whereas for TT carriers with low IP-10, SVR was 43% versus 25% with high IP-10.

Combining *IL28B* genotype and pretreatment IP-10 levels with other baseline parameters to predict treatment response

We assessed whether other baseline parameters, in addition to *IL28B* genotype and serum IP-10, could significantly improve the prediction of SVR. In this analysis, we added age, gender, race, pretreatment viral load, Ishak fibrosis score, ALT, steatosis, and histological activity index in a logistic regression model. Of all parameters included, only pre-treatment viral load (p<0.0001), *IL28B* genotype (p=0.0004), baseline IP-10 level (p=0.0033), and race (p=0.0011) significantly contributed to the model. No interaction between any pair of variables was significant (all p>0.1). When all variables were treated as categorical variables (eg, IP-10 above or below 600 pg/ml, rather than as a continuous variable), the resulting generalized linear model included the same four significant variables plus ISHAK fibrosis score (Figure 5).

ROC curve analyses were performed to compare the predictive power of models that include the most significant variables identified in these multivariate analyses (Figure 4). The AUC of the model that combined *IL28B* genotype and serum IP-10 (AUC 0.80) clearly outperformed the models based on the individual variables, including the model based on *IL28B* genotype alone (AUC 0.70). The addition of race and baseline viral load further improved the model, although the added gain was modest (AUC up to 0.85).

DISCUSSION

This is the first study to combine the highly useful *IL28B* genotype with baseline IP-10 levels to demonstrate an independent and additive model for predicting SVR with pegIFN and ribavirin treatment. We demonstrated that low pretreatment serum IP-10 is associated with sustained virological response in both Caucasian and African-American HCV genotype 1 patients from the VIRAHEP-C cohort. Using pretreatment serum IP-10 (< or > 600 pg/ml) as a predictive biomarker for treatment response in our cohort revealed a PPV of 69% and a NPV of 67%. These results are in line with other studies confirming that pretreatment IP-10

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is lower in patients who subsequently achieve SVR on therapy compared to non-responder patients (15–20). The prognostic utility of baseline IP-10 levels has also been confirmed in other difficult to treat populations such as HCV/HIV co-infection (15) and patients with an elevated viral load and BMI (18).

The current study greatly extends the potential clinical utility of IP-10 and refines the predictive value of *IL28B* gene polymorphisms. Based on five genome-wide association studies, single nucleotide polymorphisms predictive of both spontaneous and treatment-induced viral clearance in HCV genotype 1 have been identified near the *IL28B* gene (21, 24–27). Carriage of a C-allele at the *IL28B* gene polymorphism (rs12979860) is favorably associated with treatment response to pegIFN and ribavirin in both AA and CA patients (21). This was confirmed in our VIRAHEP-C cohort with SVR rates of 87% with CC, 50% with CT, and 39% with TT genotypes. Most striking, stratification by high or low baseline IP-10 (> or < 600 pg/ml) further improved the predictability of SVR among the genotype groups, especially in *IL28B* T-allele carriers. Multivariate analysis confirmed that *IL28B* genotype and baseline IP-10 levels are independent and additive predictors of HCV treatment response. Likewise, in a predictive model of SVR, serum IP-10 can be used as a "dynamic" variable which complements the "static" *IL-28B* genotype and further individualizes treatment response.

The polymorphisms on chromosome 19 associated with HCV treatment response are in the region which encodes the IFN-λ genes (*IL28A*, *IL28B* and *IL29*). The *IL28B* gene encodes IFN-λ-3 which has a unique signaling receptor as well as a common downstream signaling system with type I IFNs (22, 23). The role of IFN-λ in control of multiple viral infections including HCV is currently under study. IFN-λ has been used to block HCV replication *in vitro* (28), and human studies with IFN-λ-1 (*IL29*) yielded promising results as an antiviral agent against HCV with limited toxicity in phase 1B studies (29). Further research on the mechanism by which genetic variations near *IL28B* modulate innate immune responses via IFN-λ are ongoing.

It is unclear why high IP-10 levels are associated with nonresponse to HCV therapy. The IP-10 receptor (CXCR3) is upregulated on lymphocytes in chronic HCV and hepatocytes appear to be the predominant source of IP-10 in chronic infection (16, 30, 31). While intrahepatic IP-10 levels correlate with necroinflammatory changes and fibrosis in HCV (30), its role in viral clearance is less clear. Low pretreatment IP-10 levels are associated with a rapid decline in HCV viral load during the first 24–48 hours of interferon therapy (31). IP-10 gene expression is transiently elevated immediately post IFN injection in both AA and CA patients (12). In one study, the fold increase in IP-10 after the first pegIFN injection was associated with SVR (15). This is consistent with data that patients with low baseline levels of interferon-stimulated genes (ISG) appear to have a more robust response to exogenous pegIFN and a higher SVR rate. In contrast, those with high baseline ISG expression appear to be refractory to further IFN signaling (13, 32). High IP-10 levels may be a marker of this refractory state, or excess IP-10 may directly interfere with critical signaling pathways. Baseline hepatic ISG levels have been correlated with *IL28B* polymorphisms and treatment outcomes (33). In vivo, type III interferon IFN-λ-1 can induce IP-10 mRNA expression from PBMC in the absence of other stimuli and independent of type I IFNs (34). There may be a relationship between elevated IP-10 levels and resistance to antiviral effects of type I and type III IFNs which warrants further study.

Interestingly, our data show that at a given pretreatment IP-10 level, the probability of being a responder is also further determined by race. Race has an additive effect on the predictive models of both serum IP-10 and *IL28B* genotype but there is no statistical interaction between race, IP-10 and IL-28B (although allele frequency is race-dependent). This is in line

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with the observation that AA are generally less responsive to pegIFN and ribavirin treatment when compared to CA and that *IL28B* polymorphisms are not the only factor involved in treatment failure. Our findings confirm those of Butera et al that AA patients with chronic HCV have higher pretreatment IP-10 levels when compared to CA patients, albeit in a much smaller sample than our cohort (16). We did note that only 9% of our AA patients were *IL28B* genotype CC although the additive value for pretreatment IP-10 levels were most pronounced in the CT and TT genotypes in the combined cohort. A potential limitation of our study is that we only included sustained responders and non-responders to treatment, and no patients who relapsed after discontinuation of antiviral therapy (approximately 20% of the treatment population). However, in a previous study no distinct baseline IP-10 pattern associated with viral relapse was identified (16).

In summary, our study may have an impact on how patients with genotype 1 HCV are stratified before starting combination antiviral therapy. *IL28B* genotyping is currently being evaluated in prospective studies including triple therapy with small antiviral molecules combined with pegIFN and ribavirin. Our data show that pretreatment serum IP-10 is a strong positive predictor of SVR in both AA and CA genotype 1 patients, and significantly increases the predictive value of *IL28B* genotyping especially with the CT and TT genotypes. These two markers may prove useful in future algorithms for HCV treatment as pegIFN and ribavirin, both immune modulators, remain the backbone of therapy even with addition of small antiviral molecules. Patients lacking *IL28B* C-allele(s) (rs12979860) in combination with high IP-10 levels may require alteration of therapy type and/or duration. The utility of combining *IL28B* genotyping and baseline serum IP-10 levels to predict SVR warrants prospective validation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

HCV	Hepatitis C
pegIFN	peginterferon
SVR	sustained virological response
BMI	body mass index
AA	African-Americans
CA	Caucasian Americans
VIRAHEP-C	The Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C
IFNγ	interferon-γ
IP-10	interferon-γ inducible protein-10
ALT	alanine transaminase
PPV	positive predictive value
NPV	negative predictive value
ISG	interferon-stimulating genes

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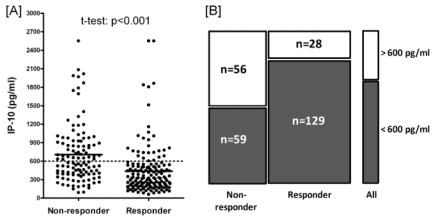

Figure 1.

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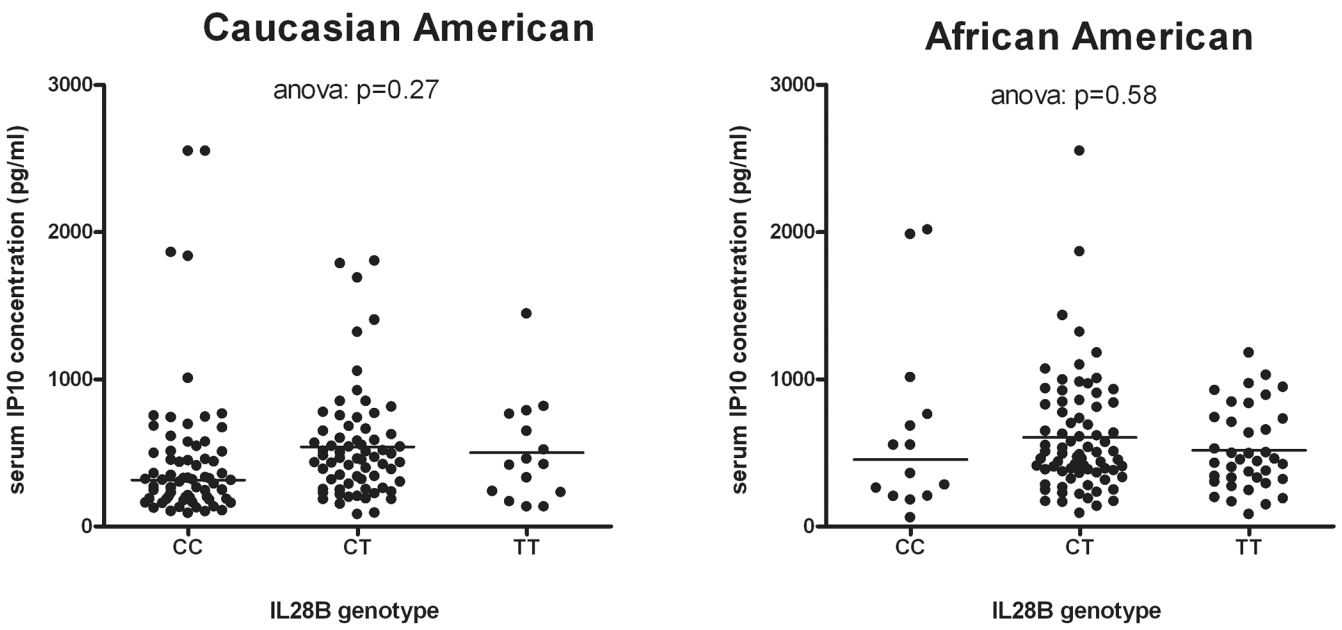

Figure 2.

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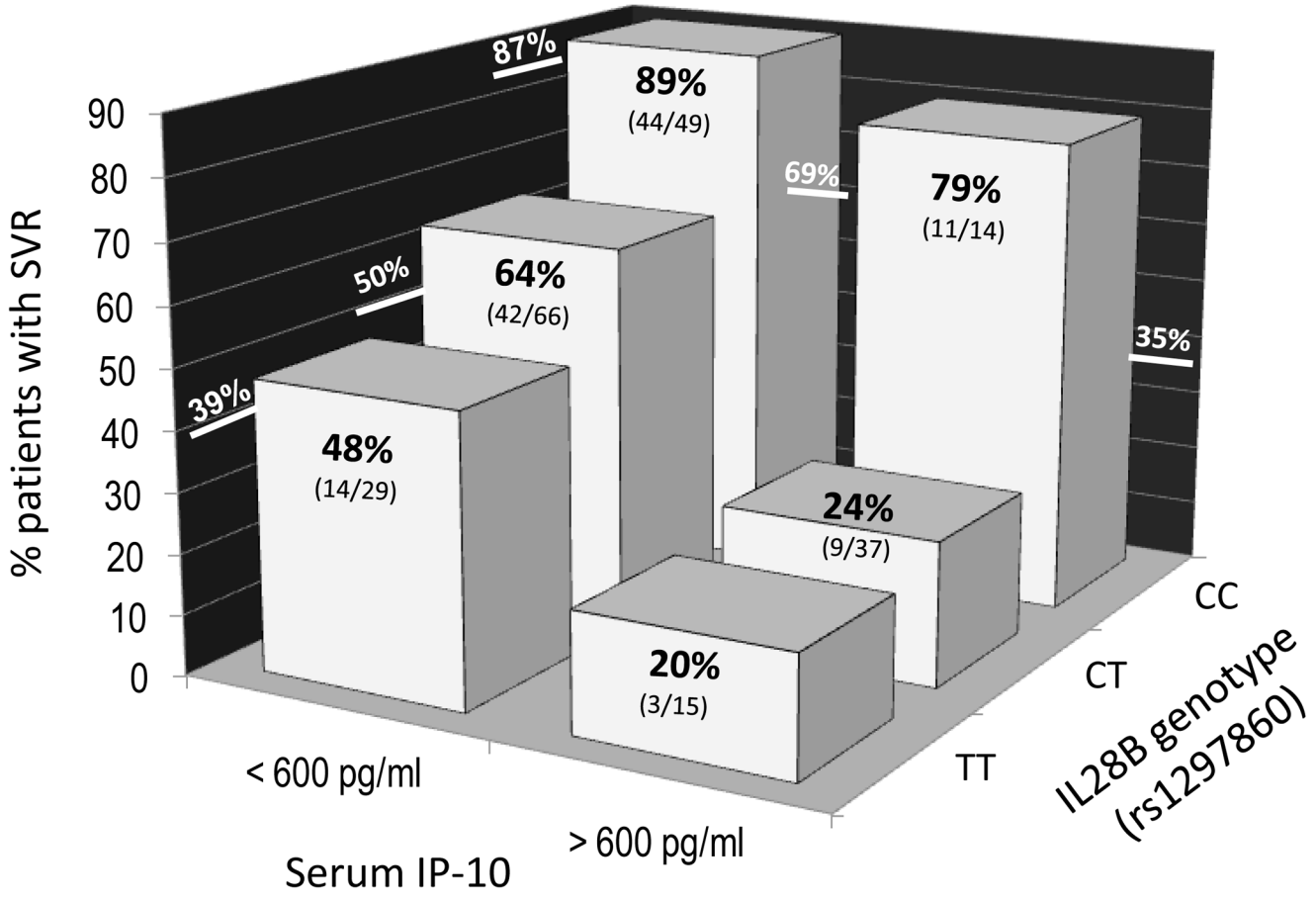

Figure 3.

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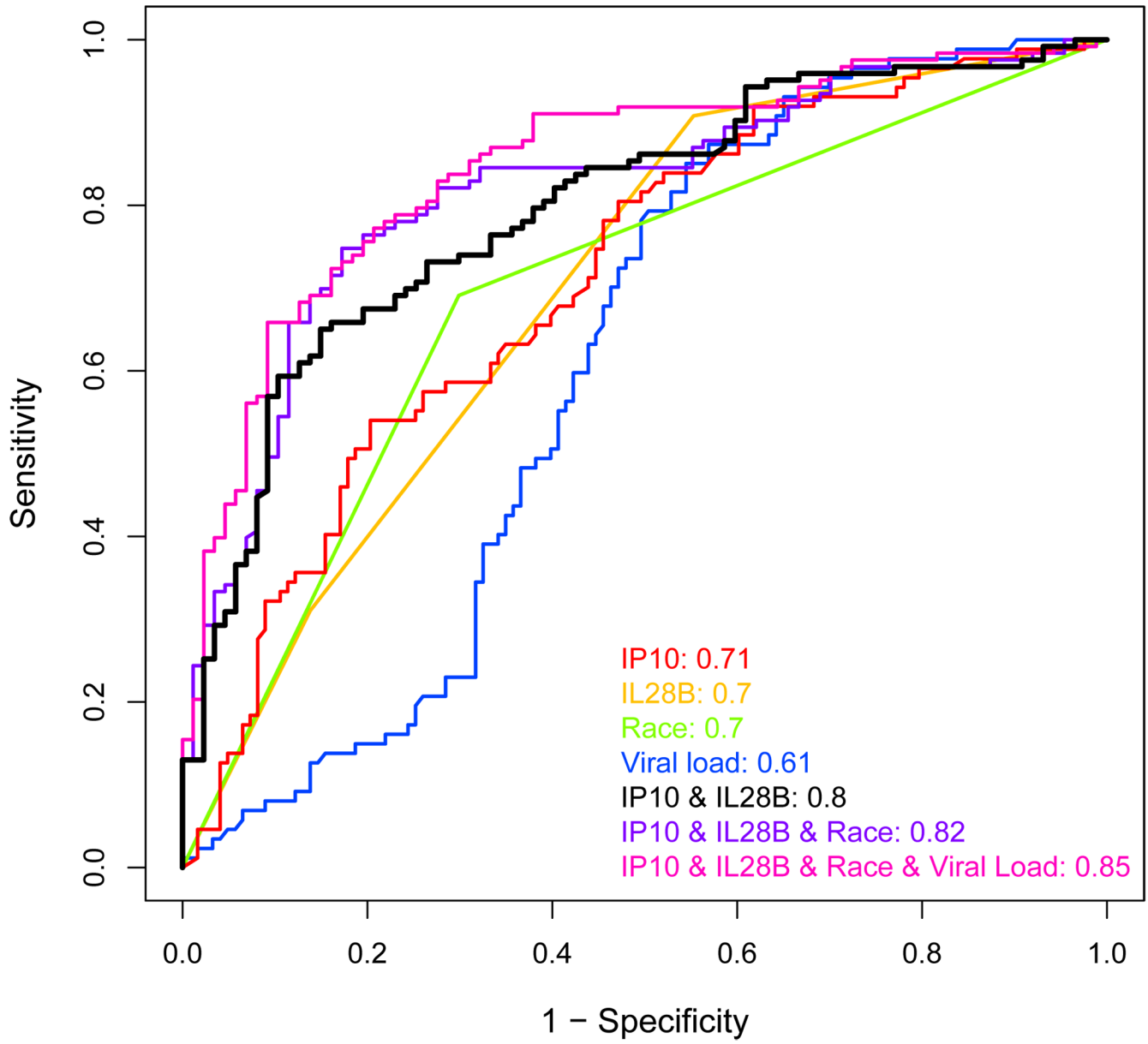

Figure 4.

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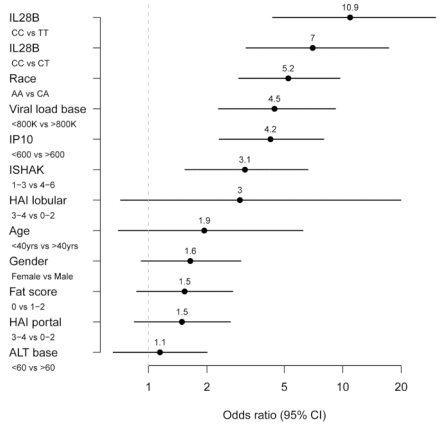

Figure 5.

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TABLE 1

Pretreatment serum IP-10 levels (pg/ml) in HCV patients stratified according to race and interferon treatment response (SVR). A significant difference was found between CA and AA for the whole cohort (p=0.015), but not within the subgroups of responders or non-responders.

	All	SVR	Non-responder	t-test
All		437 ± 31 (n=157)	704 ± 44 (n=115)	p<0.001
Caucasian	504 ± 38 (n=138)	447 ± 44 (n=104)	677 ± 69 (n=34)	p<0.001
African-American	598 ± 38 (n=134)	418 ± 35 (n=53)	716 ± 55 (n=81)	p<0.001

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TABLE 2

SVR rate according to *IL28B* genotype in the entire cohort and stratified per racial group.

IL28B genotype	CC	CT	TT	Likelihood Ratio Chi^2
% SVR entire cohort (n SVR / total n)	87% (55/63)	50% (51/103)	39% (17/44)	p < 0.0001
% SVR CA (n SVR CA / total n CA)	91% (49/54)	67% (31/46)	45% (5/11)	p = 0.0008
% SVR AA (n SVR AA / total n AA)	67% (6/9)	35% (20/57)	36% (12/33)	p = 0.20

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TABLE 3

SVR rate (%) according to a combination of *IL28B* genotype and pretreatment serum IP-10 level (<600 pg/ml: low; >600 pg/ml: high), stratified per racial group. Data are expressed as % SVR (n SVR / total n). Likelihood ratio Chi^2 analyses were performed to test for response homogeneity across the sample groups.

	Caucasian-American		African-American	
IP-10	**low**	**high**	**low**	**high**
IL28B				
CC	91% (40/44)	90% (9/10)	80% (4/5)	50% (2/4)
CT	79% (26/33)	38% (5/13)	48% (16/33)	17% (4/24)
TT	62% (5/8)	0% (0/3)	43% (9/21)	25% (3/12)
	$Chi^2 = 25.5$, $p<0.0001$		$Chi^2 = 11.8$, $p=0.037$	