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IL28B Polymorphism Is Not Associated With HCV Protease Diversity in Patients Co-Infected With HIV and HCV Treated With Pegylated Interferon and Ribavirin

Anu Osinusi^{1,2,*}, Aarthi Chary^{3,4}, Mark A. Winters^{3,4}, Susanna Naggie⁵, Henry Masur⁶, Michael A. Polis², Shyam Kottlil², and Mark Holodniy^{3,4}

¹CMRP, SAIC-Frederick, Inc., NCI-Frederick, Frederick, Maryland ²Laboratory of Immunoregulation, National Institute of Allergy and Infectious Disease, NIH, Bethesda, Maryland ³Veterans Affairs Palo Alto Health Care System, Palo Alto, California ⁴Stanford University, Stanford, California ⁵Duke Clinical Research Institute, Durham, North Carolina ⁶Critical Care Medicine Department, NIH, Bethesda, Maryland

Abstract

Recent studies have demonstrated that *IL28B* polymorphisms predict therapeutic responses in chronic hepatitis C virus (HCV)-treated patients; however, the effect on HCV viral diversity, particularly on the HCV protease gene, is not clear. This study sought to evaluate the effect of *IL28B* polymorphisms on HCV diversity at NS3/4 protease region, which may influence therapeutic response to an HCV protease inhibitor based regimen. Twenty-two patients co-infected with HIV and HCV genotype 1, treatment-naïve on stable HIV antiretroviral therapy initiating interferon-based treatment were evaluated. Plasma HCV NS3 gene diversity was analyzed by clonal analysis at baseline and end of treatment. *IL28B* (rs12979860) genotypes were tested for associations with virologic outcomes and diversity parameters. There was similar baseline NS3 diversity in patients with CC (favorable) genotype compared to those with CT/TT (unfavorable) genotypes. There was no significant association between *IL28B* genotype and baseline NS3 nucleotide p-distance, dS-dN, amino acid p-distance, or nucleotide changes. Among patients without a sustained virologic response, between baseline and follow-up there was a significant trend towards decreased diversity after treatment among patients with favorable genotype, which was not observed in unfavorable genotypes. In patients treated with peginterferon/ribavirin therapy, *IL28B* polymorphism was not associated with enhanced NS3 diversity at baseline. Among non-SVR patients with the less favorable genotype, there was no change in diversity after treatment. This suggests that *IL28B* genotype is unlikely to have a negative impact on subsequent HCV PI efficacy in patients co-infected with HIV and HCV patients who have previously failed HCV therapy.

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*Correspondence to: Anu Osinusi, MD, MPH, Bldg10, Rm.6A-33A, 10 Centre Drive, Bethesda, MD 20892. osinusia@niaid.nih.gov. Anu Osinusi and Aarthi Chary contributed equally to this study.

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Keywords

HIV/HCV; IL28B; HCV protease diversity; genomics

INTRODUCTION

Coinfection with hepatitis C virus (HCV) occurs in approximately one-third of HIV-infected patients in the United States [Sherman et al., 2002]. Improvements in antiretroviral therapy (ART) have resulted in decline of HIV-related mortality; however, HCV-related liver disease has become a leading cause of progressive liver disease, liver transplantation, hospitalization, and death in this population [Bica et al., 2001; Weber et al., 2006].

The previous standard of care for HCV treatment of pegylated interferon-alpha (PegIFN) and ribavirin (RBV) achieved a sustained virologic response (SVR) in <50% of treated individuals. These cure rates are lower among patients co-infected with HIV and HCV and minority populations, particularly African-Americans [Backus et al., 2005; Maida et al., 2010; Hadigan and Kottlil, 2011]. Hepatitis C treatment is evolving rapidly with the approval of the first direct acting antivirals, the HCV NS3-4A protease inhibitors telaprevir and boceprevir in 2011, as these drugs have been shown to be substantially more effective in patients with HCV genotype 1 in combination with PegIFN/RBV compared to PegIFN/RBV alone [McHutchison et al., 2009; Bacon et al., 2011; Burney and Dusheiko, 2011; Poordad et al., 2011].

A major reason for differential therapeutic response rates among groups has recently been attributed to an *IL28B* polymorphism, which has been found to be strongly associated with treatment outcomes in HCV genotype-1 infected individuals treated with PegIFN/RBV [Ge et al., 2009; Tanaka et al., 2009; Thompson et al., 2010]. The rs12979860 CC (favorable) genotype is associated with a more than twofold increased rate of SVR than the CT or TT (unfavorable) genotypes, a finding that is consistent across multiple ethnic groups [Ge et al., 2009; Tanaka et al., 2009; Thompson et al., 2010] and has also been confirmed in patients co-infected with HIV and HCV [Rallon et al., 2010]. In the US, HIV/HCV co-infection occurs to a large extent in minority populations [Backus et al., 2005] among who a greater frequency of the unfavorable *IL28B* genotypes is observed [Ge et al., 2009; Thompson et al., 2010]. In registration trials of NS3 protease inhibitors, in the limited subset of patients in whom IL28B genotype results were available, there were differences in response rates between treatment naïve and treatment experienced patients with similar IL28B genotypes [Jacobson et al., 2011; Pol et al., 2011].

Earlier published studies have shown no effect of prior pegIFN/RBV or ART on NS3 diversity in co-infected patients [Chary et al., 2010; Winters et al., 2010]. Other studies have suggested that mutations in the NS3 regions of the HCV epitope might impair the induction of epitope-specific CD8(+) T cells and may contribute to viral sequence evolution in infected patients [Seifert et al., 2004; Gaudieri et al., 2006]. While in monoinfected patients the NS3 region of HCV genotype-1 has demonstrated significant natural variability at the amino acid level (up to 10–11%) and nucleotide sequence level (up to 26–30%) [Holland-Staley et al., 2002], there are no data regarding the relationships between IL28B genotype and viral factors such as NS3 diversity in patients co-infected with HIV and HCV. Yet, baseline HCV NS3 diversity may have implications for future use of drugs targeting the NS3 region. With this changing HCV treatment landscape, this study sought to evaluate the effect of IL28B polymorphisms on HCV diversity at NS3/4 protease region, and its potential to impact subsequent therapeutic response to future PI containing regimens.

METHODS

Two prospective, pilot, single center, open-label trials were performed at the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health. Twenty-two treatment-naïve genotype 1 patients co-infected with HIV and HCV on stable ART were treated with either weekly injections of pegIFN- α -2a (Pegasys, Roche Laboratories, Hoffmann-La Roche Inc) or pegIFN- α -2b (Peg-Intron; Merck Inc.) plus weight-based RBV daily (at 1,000 mg dose <75 kg, 1,200 mg dose for >75 kg).

All studies were performed in compliance with relevant laws and institutional guidelines and in accordance with the ethical standards of the Declaration of Helsinki. All patients gave written informed consent including provision of genetic material as approved by the NIAID Institutional Review Board prior to enrollment in the studies.

Outcomes were defined as SVR (undetectable HCV RNA 24 weeks after end of treatment); nonresponse (NR: detectable HCV RNA through week-24 or a <2 log₁₀ copy/ml reduction in HCV RNA at week 12 of treatment), or relapse (REL; decrease in HCV RNA to undetectable levels during treatment but subsequent increase to detectable levels after end of treatment). HCV genotyping was performed using the Versant HCV genotype line probe assay (version 2.0; Siemens Healthcare Diagnostics). Plasma HIV and HCV-RNA loads were quantified using the Versant HIV RNA assay (version 3.0; Siemens Healthcare Diagnostics; lower limit of detection, 50 copies/ml) and the Abbott HCV assay (Abbott laboratories), respectively.

IL28B Genotyping

The cohort was tested for the *IL28B* SNP rs12979860 on chromosome 19 in a blinded fashion on DNA specimens collected from each individual, using the 5' nuclease assay with allele specific TaqMan probes (ABI TaqMan allelic discrimination kit and the ABI7900HT Sequence Detection System (Applied Biosystems, Carlsbad, CA). Genotyping calls were manually inspected and verified prior to release.

HCV NS3 Diversity Testing

Patients were evaluated for HCV quasispecies diversity at baseline (prior to initiation of interferon-based therapy) and at a follow-up time point no more than 4 weeks after completion of HCV therapy. HCV NS3 sequences from each patient were generated; approximately 20 clones per sample per time point were sequenced. The reverse-transcription polymerase chain reactions generated amplicons spanning nucleotides 3,315–4,298 of the HCV genome (National Center for Biotechnology Information Reference Sequence NC_004102). Diversity, including ratios between synonymous and non-synonymous nucleotide substitutions (dS/dN), p-distance and normalized Shannon entropy, was analyzed using Molecular Evolutionary Genetics Analysis software (version 4), as described in detail elsewhere [Tamura et al., 2007; Winters et al., 2010].

Statistical Analysis

For descriptive statistics, continuous variables were reported as median (25th to 75th percentiles). Categorical variables were represented as frequencies and percentages. Comparisons within groups (paired comparisons) or between groups (unpaired comparisons) were determined using Wilcoxon-signed rank tests and Mann–Whitney's *U*-tests, respectively. Categorical measures between groups were determined using the Chi-square test. All reported *P*-values are two-tailed, and results for which *P* < 0.05 were considered statistically significant.

RESULTS

Twenty-two genotype 1 patients co-infected with HIV and HCV had *IL28B* genotyping and diversity data available. Their baseline demographics are presented in Table I. *IL28B* genotype was favorable in 8 (36%), CT 8 (36%) and TT 6 (27%) patients, respectively. Patients were predominantly male (91%), mean age of 44 years, with 50% Caucasian. Nine (41%) patients had a SVR and 13 (59%) were characterized as NR/REL. Patients with favorable genotype and unfavorable genotypes were similar with respect to age, race, gender, fibrosis, baseline HCV viral loads, and CD4 cell counts. There was a significant difference in early virologic response (EVR) between favorable and non-favorable genotypes (100% vs. 43%, $P < 0.015$) and there were no favorable genotype patients that were NR (0% vs. 28% $P = 0.094$, Table I).

PI resistance mutations seen in this cohort included V36M, R155G, and A156V. Prevalence of these minor NS3 mutations was low, and occurred both at baseline and, in the nonresponse/relapse group, at follow-up. In the nonresponse/relapse group, two patients were found to have a single clone with the R155G mutation at baseline only, and each of these patients also had a different single clone at baseline with another NS3 mutation (P88L and A156V). Two different patients had the R155G substitution at follow-up. In the SVR group, a V36M mutation was found in all of the baseline clones from one patient. Otherwise, no other major NS3 mutations were noted, including no substitutions at active site positions or metal-binding residues, and the observation or frequency of NS3 mutations was not associated with *IL28B* genotype.

Genetic diversity and complexity measures were calculated from nucleotide sequences of 903 NS3 gene clones (median 22 clones per patient time-point; range 13–30 clones). There was no difference in the number of clones analyzed per patient per time-point between the two groups or between the two time-points within the two groups. All clones clustered phylogenetically appropriately by patient, and all patients' sequences segregated appropriately by genotype.

There was similar baseline NS3 diversity in patients who were favorable genotype compared to those that were unfavorable genotypes (median genetic distance = 0.011 vs. 0.006 nucleotides; median dS/dN = 0.029 vs. 0.020 nucleotides; amino acid (aa) P -distance = 0.005 vs. 0.004 in favorable vs. unfavorable genotypes, $P > 0.05$; Table II). When baseline and follow-up diversity among nonresponders and relapsers (NR/REL) was evaluated, there was no significant change in diversity at the nucleotide or amino acid level over time ($P > 0.05$; Table II). There was, however, a significant decrease in diversity over time among nonresponder and relapsed patients with favorable genotype at the nucleotide level (baseline vs. follow-up: median genetic distance 0.011 vs. 0.005 nucleotides, $P = 0.04$; median nucleotide base difference per sequence 5.621 vs. 2.774, $P = 0.02$). A similar trend was also observed in diversity at the amino acid level over time among nonresponder and relapsed patients with favorable genotype though not statistically significant (baseline vs. follow-up, median P -distance at the amino acid level 0.005 vs. 0.002 amino acids, $P = 0.08$; median dS/dN 0.029 vs. 0.014 amino acids, $P = 0.08$). In contrast, there were no changes observed in diversity over time among nonresponder and relapsed patients with unfavorable genotypes ($P > 0.05$).

While our previous studies have found significant differences in baseline diversity based on treatment outcome in this same population [Chary and Holodniy, 2010], among this smaller study subset there were no significant differences in baseline diversity based on subsequent treatment outcomes.

DISCUSSION

This study evaluates the relationship of an *IL28B* polymorphism and HCV NS3 sequence diversity in HIV co-infected patients. Our results indicate there is no significant effect of rs12979860 *IL28B* polymorphism on baseline NS3 quasispecies diversity in co-infected subjects. Major NS3 PI resistance or active site NS3 mutations were also not observed frequently in either group. Furthermore, among the nonresponder and relapsed patients that were unfavorable genotype, there was no change in diversity from baseline over time. These results suggest that this *IL28B* polymorphism would not markedly impact the potential efficacy of subsequent HCV PI treatment in co-infected patients, even among those who have failed prior HCV treatment with peg-IFN/RBV.

Previous work demonstrated a significant association between HCV treatment failure and higher baseline NS3 diversity in this cohort of patients co-infected with HIV and HCV. While a similar trend was also noted in this study, it did not reach statistical significance in the current analysis likely due to a smaller sample size. Prior studies have demonstrated correlations exist between increased quasispecies variability in the HCV hypervariable region 1 (HVR1) at baseline and a lack of response to antiviral therapy in genotype 1 mono-infected patients [Morishima et al., 2006; Shire et al., 2006; Salmeron et al., 2008]. Some studies have shown low baseline HVR1 genetic complexity, in conjunction with other factors predict interferon response, whereas other studies have found a correlation of quasispecies complexity with early, but not sustained, virologic response [Abbate et al., 2004]. This has also been observed in interferon-treated HIV co-infected subjects [Sherman et al., 2010; Shire et al., 2006]. These studies suggest that variations in quasispecies occurring in the absence of drug pressure may be due to host immune response and other factors, which are yet to be fully elucidated.

PI resistance mutations seen in our study included V36M, R155G, and A156V, which have been reported in both boceprevir and telaprevir trials [Hezode et al., 2009; Pawlotsky, 2009; Kwo et al., 2010]. These mutations are known to confer varying levels of resistance to HCV protease inhibitors [Kieffer et al., 2007; Curry et al., 2008; Pawlotsky, 2009]. These NS3 substitutions were seen at similar frequency both at baseline and at follow-up among those with nonresponse/relapse. Overall, protease inhibitor resistance mutations were infrequently observed in this cohort and were not associated with *IL28B* genotype.

Limitations of this study include the small sample size in each group and the absence of nonresponders within the favorable genotype and as were therefore unable to evaluate NS3 diversity differences between genotype 1a and 1b, or race and gender effects. This study was also unable to evaluate the relationship between *IL28B* genotype and HCV protease inhibitors combined with pegIFN/RBV on NS3 diversity and subsequent virologic failure.

While the current study failed to find a significant correlation between baseline diversity and *IL28B* genotype, this data suggest that NS3 diversity may decrease with treatment among patients with *IL28B* favorable genotype but not the unfavorable genotypes; this difference in change in diversity during treatment may be a marker of host immune response or selection pressures related to *IL28B* genotype and warrants further study. Larger studies in both HCV mono-infected and patients co-infected with HIV and HCV will be needed to elucidate further the immunologic mechanisms by which host *IL28B* impacts treatment response.

While likely that overall change in HCV NS3 diversity after pegIFN/RBV treatment is minimal, *IL28B*-related immune effects resulting in even minor changes in NS3 variants may favorably or negatively impact subsequent re-treatment with PI-based therapy. As HCV protease inhibitors become the new standard of care in clinical practice, further

understanding of HCV NS3 diversity and the potential impact on treatment in this challenging population is needed.

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TABLE I**Clinical Characteristics of Patients**

Baseline characteristic	CC favorable genotype (N = 8)	CT/TT unfavorable genotype (N = 14)	P-value*
Age (years)	41 (38,41)	43.5 (41,51)	0.3782
Male gender	7 (87.5)	13 (92.8)	0.6742
Non-white race/ethnicity	6 (75)	5 (36)	0.0763
BMI	24.2 (22.8, 25.9)	27.4 (24.6, 29.0)	0.076
HCV RNA (log10 IU/ml)	6.35 (5.84, 6.69)	6.304 (5.87, 6.51)	0.8645
HCV RNA >800,000 IU/ml	6 (75)	11 (78.6)	0.8475
HAI fibrosis stage			
F0–F2	5 (62.5)	10 (71.4)	0.6645
F3–F4	3 (37.5)	4 (28.6)	
Interferon formulation			
2b 1.5 µg/kg/week	7 (87.5)	9 (64)	0.2396
2a 180 µg/week	1 (12.5)	5 (36)	
Baseline CD4 cells/µl	678 (537,849)	475 (296,705)	0.1721
HIV RNA IU/ml	<50 (<50, 536)	<50 (<50, 1316)	0.5659
Suppressed HIV RNA	6 (75)	8 (57)	0.4023
EVR	8 (100)	7 (50)	0.0154*
NR	0	4 (28)	0.094
SVR	3 (37.5)	6 (42.8)	0.8085

EVR, early virologic response; NR, nonresponse; SVR, sustained virologic response.

* *P*-value < 0.05 is considered significant.

Values are median (interquartile ranges) or (percentages). *P*-values: chi-square or Mann–Whitney's *U*-tests.

TABLE II

Comparison of Median HCV NS3 Gene Quasispecies Parameters in Patients Co-Infected With HIV and HCV Based on IL28B Genotype

Parameter	CC favorable genotype (N = 8)				CT/TT unfavorable genotypes (N = 14)				P (comparison of baseline CC vs. CT/TT)
	Baseline (n = 8)	Follow-up (n = 5)	P (NR/REL only) ^a		Baseline (n = 14)	Follow-up (n = 8)	P (NR/REL only) ^a		
Genetic distance (range), nucleotides	0.011 (0.002–0.034)	0.005 (0.004–0.007)	0.043 *		0.006 (0.001–0.024)	0.010 (0.001–0.028)	1.0		0.34
dS/dN (range), nucleotides	0.029 (0.005–0.133)	0.014 (0.010–0.025)	0.08		0.020 (0.002–0.100)	0.029 (0.001–0.098)	0.48		0.45
No. of base differences per sequence	5.621 (1.251–18.240)	2.774 (2.238–3.944)	0.016 *		3.091 (0.628–13.000)	5.158 (3.375–15.183)	0.86		0.21
P-distance (range), amino acids	0.005 (0.002–0.017)	0.002 (0.001–0.005)	0.08		0.004 (0.000–0.010)	0.005 (0.000–0.020)	1.0		0.16

NR/REL, nonresponders/relapsers.

^aWilcoxon-signed rank test used for paired comparisons between baseline and follow-up diversity among NR/R; Mann-Whitney's U-test used for baseline comparisons between CC favorable genotype and CT/TT unfavorable genotypes.

* Significant P-value at 0.05.