

HCV RNA Levels in a Multiethnic Cohort of Injection Drug Users: Human Genetic, Viral and Demographic Associations

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In patients with chronic hepatitis C, the hepatitis C virus (HCV) RNA level is an important predictor of treatment response. To explore the relationship of HCV RNA with viral and demographic factors, as well as *IL28B* genotype, we examined viral levels in an ethnically diverse group of injection drug users (IDUs). Between 1998 and 2000, the Urban Health Study (UHS) recruited IDUs from street settings in San Francisco Bay area neighborhoods. Participants who were positive by HCV enzyme immunoassay were tested for HCV viremia by a branched-chain DNA assay. HCV genotype was determined by sequencing the HCV nonstructural 5B protein region. For a subset of participants, *IL28B* rs12979860 genotype was determined by Taqman. Among 1,701 participants with HCV viremia, median age was 46 years and median duration of injection drug use was 26 years; 56.0% were African American and 34.0% were of European ancestry (non-Hispanic). Human immunodeficiency virus type 1 (HIV-1) prevalence was 13.9%. The overall median HCV RNA level was 6.45 log₁₀ copies/mL. In unadjusted analyses, higher levels were found with older age, male gender, African-American ancestry, hepatitis B virus infection, HIV-1 infection, and *IL28B* rs12979860-CC genotype; compared to participants infected with HCV genotype 1, HCV RNA was lower in participants with genotypes 3 or 4. In an adjusted analysis, age, gender, racial ancestry, HIV-1 infection, HCV genotype, and *IL28B* rs12979860 genotype were all independently associated with HCV RNA. **Conclusion: The level of HCV viremia is influenced by a large number of demographic, viral, and human genetic factors. (HEPATOLOGY 2012;56:86-94)**

Chronic infection with hepatitis C virus (HCV) is a leading cause of hepatocellular carcinoma, end-stage liver disease, and liver transplantation.¹ Successful antiviral treatment (i.e., sustained

virological response; SVR) reduces the risk of these outcomes. Higher HCV RNA levels are associated with a lower rate of SVR to current standard pegylated interferon (Peg-IFN)/ribavirin (RBV) therapy² and,

Abbreviations: ALIVE, the AIDS Linked to the Intravenous Experience Study; bDNA, branched-chain DNA assay; CD, cluster of differentiation; CHC, chronic hepatitis C; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1; IDUs, injection drug users; IFN, interferon; IL, interleukin; IQR, interquartile range; NCI, the National Cancer Institute; NS5B, nonstructural 5B protein; PCR, polymerase chain reaction; Peg-IFN, pegylated IFN; RBV, ribavirin; RT, reverse transcription; SVR, sustained virological response; UHS, the Urban Health Study.

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possibly, higher rates of maternal-fetal transmission.³ In previous studies, a number of factors have been shown to be associated with higher HCV RNA levels, including demographic, viral, and human genetic factors,⁴⁻⁷ but, to our knowledge, no previous study has looked at all of these elements simultaneously.

The incidence and prevalence of HCV infection among injection drug users (IDUs) are high. The Urban Health Study (UHS) was an epidemiological and interventional research project that enrolled a multiethnic population of IDUs in the San Francisco Bay area. Between 1998 and 2000, we collected data and specimens from these persons for studies of demographic, viral, and host determinants of infection with viruses that may cause cancer.^{8,9} At that time, this group had extremely limited access to anti-HCV therapies; therefore, HCV RNA levels in UHS participants are largely unaffected by selective effects of previous treatment. Here, we explore the association of virologic and demographic factors, as well as *IL28B* genotype, on HCV RNA levels in this multiethnic cohort of HCV-infected IDUs.

Patients and Methods

Subjects and Data Collection. As previously reported, UHS investigators recruited IDUs from six San Francisco Bay area neighborhoods.¹⁰ All individuals 18 years of age or older who had injected illicit drugs within the past 30 days or who had previously participated in UHS were eligible for enrollment. Study participants received modest monetary compensation. Although some participants had received hepatitis B vaccine,⁹ few, if any, were treated for hepatitis B virus (HBV) or HCV infection. Participants were not asked about treatment for HCV infection during 1998-2000, but in 2002, only 3% of UHS participants reported IFN-based treatment for HCV infection,¹¹ thus the vast majority of subjects in this study had never received treatment for chronic hepatitis C (CHC). Among the 237 subjects in this analysis who tested positive for human immunodeficiency virus type 1 (HIV-1), 47 (19.8%) reported taking at least one antiretroviral drug at the time of enrollment.

Trained staff obtained informed consent from the participants, including explicit written consent for host genetic testing. Participants were interviewed using a standardized instrument, counseled on reducing infection risks, and referred to appropriate medical and social services. Participants were asked about sociodemographic characteristics and their injection drug history, including age at first injection. Blood samples were collected by a trained phlebotomist. Further details about UHS are provided elsewhere.¹⁰ The study was approved by the Committee on Human Subjects Research at the University of California at San Francisco (San Francisco, CA) and an Institutional Review Board of the National Cancer Institute (NCI).

We assessed possible repeat enrollment by comparing demographic information, including gender, birth date, race, and site of enrollment. Enrollees who appeared very similar demographically were evaluated by DNA testing (as described below). Among 2,296 UHS participants, 2,092 were positive for HCV antibody, of whom 2,073 had sufficient specimen to be tested for HCV RNA. Among these 2,073 participants, 1,701 had detectable HCV RNA in the plasma and were included in the current study.

Viral Serology. As previously described,⁹ to define HCV infection status, we first tested for HCV antibody by the HCV version 3.0 enzyme-linked immunosorbent assay Test System (Ortho-Clinical Diagnostics, Raritan, NJ). Participants who were positive by HCV enzyme immunoassay were considered to have been infected with HCV and those with sufficient archived plasma ($n = 2,073$) were tested for HCV viremia by a branched-chain DNA assay (bDNA) (VER-SANT HCV RNA 3.0 Assay, analytic sensitivity 2.5×10^3 copies/mL; Bayer-Diagnostics, Tarrytown, NY). Those positive for HCV RNA were considered to have chronic HCV infection and those with a negative result were considered to have resolved HCV infection. Methods of testing for HIV-1 and HBV infection status in these subjects have been described.⁹

Determination of Viral Genotypes. Total nucleic acid was isolated from 500 μ L of serum (Roche MagNa Pure LC Total Nucleic Acid Isolation Kit-

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Additional Supporting Information may be found in the online version of this article.

Large Volume; Roche Diagnostics Corporation, Indianapolis, IN), and reverse transcription (RT) was performed. Polymerase chain reaction (PCR) was carried out in a reaction mixture containing 3 μ L of complementary DNA, 10 μ L of HotStar Taq Master Mix (Qiagen, Valencia, CA), and 1 μ L of each of the following primers: forward 5'-TGGGGTTCTC GTATGATACCC-3' and reverse 5'-CCTGGTCATAG CCTCCGTGAA-3', to amplify the 5'-NS5B (non-structural 5B protein) region. PCR product was purified with Exosap-IT (USB Corporation, Cleveland, OH) and combined with 2.0 μ L of Big Dye terminator (ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit v3.1; Applied Biosystems, Foster City, CA) and 100 pmol of primer forward (5'-NC or 5'-NS5B). The sequencing reaction was carried out for 30 cycles, and electrophoresis was performed on an ABI Prism 3730 XL instrument (Genewiz, South Plainfield, NJ).

Raw sequence data were analyzed by Sequencher 4.8 Gene codes to trim ambiguous sequences. To query HCV genotype, sequences were compared to an HCV database operated by the Los Alamos National Laboratory (available at: http://hcv.lanl.gov/content/sequence/BASIC_BLAST/basic_blast.html) using BLAST. Viral genotype call was based on the highest score and lowest e-value, using the NS5B sequence, unless those results were negative or missing, in which case genotype was based on the 5'NC region.

IL28B Genotyping. DNA was extracted from cryopreserved lymphocytes using a modified salt precipitation-extraction method (Gentra Systems, Minneapolis, MN) or from granulocytes using a silica membrane-binding method using Qiagen DNA purification columns (Qiagen). The NCI Core Genotyping Facility performed genotyping for *IL28B* rs12979860 using an optimized TaqMan assay (available at: <http://variantgps.nci.nih.gov/cgfsseq/pages/snp500.do>).

Statistical Analyses. All analyses were cross-sectional and based on a single study visit. We determined median HCV RNA levels (\log_{10} copies/mL) overall and among subgroups. As neither \log_{10} HCV RNA nor alternative transformations of these data were normally distributed, nonparametric statistical methods were the basis of the analysis. We used the Wilcoxon (Kruskal Wallis) test to compare the distribution of HCV RNA levels for variables in SAS PROC NPARIWAY. To perform multivariate analysis, we divided HCV RNA into quintiles and examined determinants of higher HCV RNA in unconditional ordinal logistic regression models that included age (or duration of injection drug use), gender, race/ethnicity, HBV infection, HIV-1 infection, and HCV genotype

(SAS PROC LOGISTIC). All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

Results

Study Population. A total of 2,092 UHS subjects had antibody to HCV. Among these, 2,073 participants had sufficient plasma to be tested for HCV RNA, of whom 1,701 (82.1%) had detectable HCV RNA. Demographic and clinical features for the 1,701 participants with HCV viremia were generally similar to those among all UHS subjects with HCV antibodies (Table 1). Among those with detectable HCV RNA, median age at enrollment was 46 years, median age at which a drug was first injected was 18 years, and median time from first use of injection drugs to enrollment was 26 years. Most participants (72.4%) were men. Over half (56.0%) of the participants considered themselves African American, 34.0% white (non-Hispanic), 6.8% Latino (non-African American), 1.1% were Asian or Pacific Islanders, and 2.2% were American Indian or Alaska natives. Infection with HIV-1 was present in 237 (13.9%) participants. As previously reported in this and other cohorts,^{7,8,12} chronic hepatitis B was less frequent and HIV-1 infection was more frequent among participants with CHC.

Predictors of HCV RNA Levels. All subjects. Among participants with detectable virus, median HCV RNA level was 6.45 \log_{10} copies/mL (interquartile range [IQR], 5.97-6.89). Median viral levels were progressively higher in each older age category, ranging from 6.15 \log_{10} copies/mL among participants 18-29 years at enrollment to 6.59 \log_{10} copies/mL among those >50 years of age ($P < 0.0001$; Table 2). Duration of injection drug use is highly correlated with age at enrollment in UHS participants ($r^2 = 0.74$), and there was also a strong trend toward higher HCV RNA levels with longer duration of drug use (<0.0001). HCV RNA levels were higher in men (6.52 \log_{10} copies/mL) than women (6.29 \log_{10} copies/mL; $P < 0.0001$). With regard to race and ethnicity, the highest levels were found in African-American participants (6.49 \log_{10} copies/mL), intermediate viral levels were found in white participants of non-Hispanic origin (6.35 \log_{10} copies/mL) and Latinos (6.39 \log_{10} copies/mL), and the lowest levels were found in those who reported their ancestry as Asian, Pacific Islander, American Indian, or an Alaska native (6.24 \log_{10} copies/mL; Table 2), with similar median HCV RNA levels among Asian/Pacific Islanders (6.24 \log_{10} copies/mL; $n = 19$) and American Indians/Alaska natives (6.18 \log_{10} copies/mL; $n = 37$). Regarding infection with other viruses, for subjects overall,

Table 1. Characteristics of HCV-Infected IDUs, San Francisco Bay Area, 1998-2000

Characteristic	HCV Antibody (n = 2,092)		HCV Viremia (n = 1,701)		Viral Genotype Determined (n = 1,524)		IL28B Genotype Determined (n = 882)	
	Median	IQR*	Median	IQR	Median	IQR	Median	IQR
Age at enrollment	45	40-50	46	40-50	46	40-50	44	39-49
Years of injection drug use	25	17-31	26	18-32	26	18-32	25	18-31
Age at first injection drug use	18	16-24	18	16-24	18	16-24	18	16-22
	No.	%	No.	%	No.	%	No.	%
Gender†								
Male	1,461	70.9	1,222	72.4	1,104	73.1	615	69.7
Female	600	29.1	465	27.6	407	26.9	267	30.3
Race								
White	759	36.3	577	33.9	522	34.3	391	44.3
African American	1,078	51.5	951	55.9	861	56.5	347	39.3
Latino	167	8.0	115	6.8	92	6.0	88	10.0
Asian or Pacific Islander	28	1.3	19	1.1	15	1.0	18	2.0
American Indian	58	2.8	37	2.2	32	2.1	36	4.1
Other	2	0.1	2	0.1	2	0.1	2	0.2
HBV infection								
Acute	18	0.9	7	0.4	7	0.5	5	0.6
Chronic	53	2.5	29	1.7	26	1.7	28	3.2
Resolved	1,641	78.4	1,348	79.2	1,197	78.5	680	77.1
Vaccinated	78	3.7	70	4.1	65	4.3	38	4.3
Uninfected and unvaccinated	302	14.4	247	14.5	229	15.0	131	14.9
HIV-1 infection	262	12.5	237	13.9	211	13.8	107	12.1

*25th percentile to 75th percentile.

†Missing data: gender, 31; HIV-1 infection, 3.

those without antibody to HBV had lower HCV RNA levels than those with resolved or chronic HBV infection (Table 2). HCV RNA levels were higher in HIV-infected participants (6.73 log₁₀ copies/mL) than in HIV-uninfected IDUs (6.40 log₁₀ copies/mL; $P < 0.0001$).

We also performed analyses stratified by HIV-1 infection status (Table 2). Among the HIV-1-uninfected participants, the patterns of association were very similar to those observed among all viremic subjects, except that the HCV RNA level was consistently lower for each characteristic examined. Among the 237 HIV-1-infected participants, differences by age, gender, and duration of drug use were blunted or absent, but differences between African-American and white participants were preserved ($P = 0.01$).

Among the participants with detectable virus, 1,669 had a specimen available for viral genotyping and 1,524 (91.3%) of those subjects were successfully genotyped (Table 3). Most participants were infected with an HCV-genotype 1 strain (1a, 69.0%; 1b, 10.0%), but 9.3% were infected with a genotype 2 strain, 10.6% with a genotype 3 strain, and 1.1% with genotype 4a. Median HCV RNA level did not differ significantly between participants infected with 1a (6.50 log₁₀ copies/mL) and those infected with 1b (6.63 log₁₀ copies/mL; $P = 0.11$). In comparison to partici-

pants who were infected with genotype 1 (median HCV RNA, 6.50 log₁₀ copies/mL), HCV RNA was lower in those infected with genotype 3 (6.34 log₁₀ copies/mL; $P = 0.0003$) or 4 (6.12 log₁₀ copies/mL; $P = 0.03$). We observed the lowest median HCV RNA level (5.64 log₁₀ copies/mL) among participants who had detectable HCV RNA, but could not be genotyped.

IL28B rs12979860 Genotype. IL28B genotyping was performed for a subset of the participants with CHC (Table 4). Among 347 African-American participants, we observed no differences in viral levels by IL28B genotype. Among 391 European-American IDUs, those with the IL28-CC genotype had a higher median HCV RNA level (6.67 log₁₀ copies/mL) than those with IL28-TT (6.12; $P = 0.01$); median HCV RNA level among European-American participants with the IL28-CT genotype was 6.26 log₁₀ copies/mL. Among 88 participants of Hispanic ethnicity, median HCV RNA levels for those with the IL28-CC (6.63 log₁₀ copies/mL) and IL28-TT (6.19 log₁₀ copies/mL) genotypes were similar to those observed in European-American participants, but this difference was not statistically significant among participants of Hispanic ethnicity, which is a much smaller group.

Multivariate Analyses. Results from the multivariable ordinal logistic regression analysis (Table 5)

Table 2. Median HCV RNA Levels (log₁₀ Copies/mL) Among IDUs With Detectable HCV RNA,* by Selected Demographic and Viral Variables: San Francisco Bay Area, 1998-2000

Characteristic	All Subjects			HIV-1-Uninfected Subjects			HIV-1-Infected Subjects		
	No.	HCV RNA	P Value	No.	HCV RNA	P Value	No.	HCV RNA	P Value
Overall	1,701	6.45		1,461	6.40		237	6.73	
Age (years)									
18-29	84	6.15		76	6.11		8	6.32	
30-39	303	6.29		243	6.25		60	6.74	
40-49	845	6.47		724	6.43		119	6.73	
≥50	469	6.59		418	6.56		50	6.79	
			<0.0001			<0.0001			0.15
Years injection drug use									
0-9	176	6.08		158	6.04		18	6.42	
10-19	316	6.29		265	6.21		51	6.75	
20-29	602	6.47		504	6.44		96	6.62	
≥30	573	6.61		508	6.59		64	6.84	
			<0.0001			<0.0001			0.08
Gender									
Male	1,222	6.52		1,053	6.49		167	6.71	
Female	465	6.29		397	6.21		67	6.79	
			<0.0001			<0.0001			0.41
Race									
African American	951	6.49		806	6.45		144	6.78	
White	577	6.35	0.0003	500	6.34	0.006	76	6.56	0.01
Latino	115	6.39	0.0500	106	6.37	0.120	9	6.58	0.32
Asian or American Indian	56	6.24	0.0005	47	6.23	0.003	8	6.49	0.11
HBV infection									
Resolved	1,348	6.48		1,143	6.44		203	6.73	
Chronic	29	6.64		22	6.22		6	6.96	
			1.0000			0.260			0.17
Uninfected	247	6.30		230	6.25		17	6.77	
			0.0040			0.006			0.69

*Subjects with undetectable HCV RNA were excluded.

confirmed the unadjusted findings. HCV RNA levels were higher for older participants, men, and those infected with HIV-1. Compared to African Americans, HCV RNA levels were lower in all other ancestry groups, although this difference did not approach statistical significance for the comparison with Latinos ($P = 0.44$). Regarding viral genotype, compared to those

infected with genotype 1, participants infected with genotype 2 had higher HCV RNA ($P = 0.01$). *IL28B*-CC genotype was associated with higher HCV RNA ($P = 0.001$). A model that substituted duration of drug use for age produced similar results. In view of the findings in Table 4, we also conducted a multivariate analysis that including a term for an interaction between *IL28B*

Table 3. Median HCV RNA Levels (log₁₀ Copies/mL) Among IDUs With Detectable HCV RNA,* by HCV Genotype: San Francisco Bay Area, 1998-2000

HCV Genotype†	Number	Percent	Percent (Excludes NR)	Median	P Value‡	Median	P Value§
1a	1,052	63.03	69.03	6.50	(referent)	6.51	(referent)
1b	152	9.11	9.97	6.63	0.11		
2a	15	0.9	0.98	6.71	0.92	6.69	0.3200
2b	127	7.61	8.33	6.69	0.19		
3a	159	9.53	10.43	6.34	0.001	6.34	0.0003
3b	2	0.12	0.13	6.08	0.37		
4a	17	1.02	1.12	6.12	0.04	6.12	0.0300
Not resolved	145	8.69		5.64	<0.0001	5.64	<0.0001
Total	1,669						

Subjects with undetectable HCV RNA were excluded.

†Viral genotype is based on NS5B sequence.

‡In comparison with subjects with genotype 1a.

§In comparison with subjects with genotype 1.

Table 4. HCV RNA Levels Load Among IDUs With Chronic HCV, by *IL28B* rs12979860 Genotype: San Francisco Bay Area, 1998-2000

Genotype	African American (n = 347)			European American (n = 391)			Hispanic (n = 88)			Asian/American Indian (n = 54)		
	Number	RNA Median	P Value	Number	RNA Median	P Value	Number	RNA Median	P Value	Number	RNA Median	P Value
TT	123	6.44		41	6.12		10	6.19		7	6.28	
TC	177	6.51	0.33	185	6.26	0.34	48	6.53	0.21	20	6.27	0.44
CC	47	6.48	0.68	165	6.67	0.01	30	6.63	0.14	27	5.89	0.98

rs12979860 genotype and race/ethnicity, but this interaction was not statistically significant ($P > 0.10$).

Discussion

In this large multiracial cohort of IDUs with CHC infection, HCV RNA levels were independently associated with six factors: age, gender, racial ancestry, HIV-1 infection, HCV genotype, and host *IL28B* rs12979860 genotype.

HCV RNA levels tended to be higher with older age and longer duration of drug injection, variables that were highly correlated in this study. Average time since initiation of drug use in these IDUs is 19 years and, at least until recently, most IDUs who enrolled in the UHS became infected with HCV relatively soon after initiating drug injection.⁹ We believe therefore that reported years of injection drug practices is a reasonable proxy for the time since initial infection with HCV. Our data suggest that HCV RNA levels may increase over time. Consistent findings were previously reported in another cross-sectional study of IDUs,⁶ but results from longitudinal studies of HCV RNA are mixed. The study with the longest follow-up period (median, 9.2 years) found that HCV RNA levels increased over time,¹³ but studies based on shorter follow-up periods (average, 1-5 years), which may have lacked the statistical power to exclude modest increases, did not.¹⁴⁻¹⁶ We speculate that HCV may propagate more efficiently over time, perhaps because of selection of HCV variants with high replicative efficiency or host loss of immunological control of HCV.

In the absence of HIV-1 infection, HCV-RNA levels tended to be lower for women, compared to men, and this difference remained after potential confounding variables were considered. Among the 237 HIV-infected UHS participants, however, median HCV RNA levels were similar in women and men. In the AIDS Linked to the Intravenous Experience (ALIVE) study of IDUs, lower HCV RNA levels were observed in women, compared to men, among HIV-uninfected subjects, although that association was not statistically significant in a multivariate analysis.⁶ As in our study, HCV RNA levels did not differ by gender among the

HIV-infected ALIVE participants. Among HCV-infected Alaska natives, women had much lower levels of HCV RNA than men.¹⁷

Comparing HCV RNA by racial ancestry, African-American UHS participants tended to have higher levels than participants of European or Asian ancestry, even after we considered other factors, including *IL28B* genotype. Few previous studies have been able to make such comparisons. In ALIVE, no difference in HCV RNA levels was observed between African-American subjects and those of other races; however, only 40 non-African-American subjects were included in that analysis.⁶ Among patients enrolled in treatment trials for CHC, pretreatment HCV RNA levels did not differ between African-American and European-American subjects in either the Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C¹⁸ or the Initiating Dialysis Early and Late¹⁹ studies.

HCV-RNA levels were considerably higher among UHS participants who were infected with HIV-1,

Table 5. Multivariate Ordinal Logistic Regression Analysis of HCV RNA Levels (log₁₀ Copies/mL) Among 874 IDUs With CHC: San Francisco Bay Area, 1998-2000

Characteristic	Subjects With <i>IL28B</i> Genotype (n = 874)		
	OR	95% CI	P Value
Age	1.28	1.10-1.50	0.001
Female gender	0.73	0.56-0.95	0.02
Race/ethnicity			
African American	1.00	(referent group)	
White	0.70	0.52-0.94	0.02
Latino	0.84	0.54-1.31	0.44
Asian or American Indian	0.39	0.23-0.69	0.001
Chronic HBV	1.06	0.53-2.14	0.86
HIV-1	1.95	1.34-2.83	0.0004
HCV genotype			
1a/1b	1.00	(referent group)	
2a/2b	1.78	1.14-2.77	0.01
3a/3b	0.80	0.55-1.17	0.25
4a	0.37	0.12-1.15	0.09
Not resolved	0.17	0.11-0.28	<0.0001
<i>IL28B</i> genotype			
TT		(referent group)	
CT	1.10	0.80-1.51	0.17
CC	1.69	1.17-2.44	0.001

The analysis is limited to subjects with results for *IL28B* rs12979860 genotype.

compared to those who were not (6.73 versus 6.40 log₁₀ copies/mL), which is consistent with the results from a number of previous studies.^{6,13,20-33} In our study, we were able to control for a fuller range of potential confounding, but this association remained strong even when these factors were considered.

Among the subjects for whom we could determine viral genotype, almost 80% were infected with HCV genotype 1A or 1B; the median HCV RNA level in this group was 6.51 log₁₀ copies/mL. Nonetheless, consistent with other studies among IDUs,^{6,7} we found a diversity of HCV genotypes in this population: 321 UHS participants had HCV genotypes 2, 3, or 4. Those who were infected with HCV genotype 2 had higher HCV RNA levels (median, 6.69 log₁₀ copies/mL) than those infected with genotype 1, although this difference reached statistical significance only in the subsample with *IL28B* genotype data available. We observed lower viral levels in participants who were infected with genotype 3 (median, 6.34 log₁₀ copies/mL). Those findings remained significant in the multivariable analysis of the whole sample, but lost significance when the analysis was restricted to the subsample with *IL28B* genotype data, perhaps because of insufficient statistical power. Among the 17 subjects with HCV genotype 4 infection, median HCV RNA level was 6.12 log₁₀ copies/mL. Consistent with our findings, an earlier report of Swiss blood-transfusion recipients coinfecting with HIV-1 showed the highest HCV RNA levels in patients with genotype 2 and the lowest levels in patients with genotype 4.²⁴ In a multinational study (predominantly IDUs), HCV RNA levels were lowest among subjects infected with genotypes 3 or 4 and similar among those with genotypes 1 and 2, although relatively few subjects with genotype 2 were included in this analysis.⁷ Among Alaska natives, the lowest HCV RNA levels were found in persons infected with HCV genotype 3a and the highest in those infected with genotype 2b. In that population, no patients were found to be infected with genotype 4.¹⁷

Several variables that we found to be associated with higher HCV RNA among UHS participants (e.g., older age, male gender, African ancestry, and HIV infection) were previously associated with failure to spontaneously clear HCV infection in this cohort,⁸ as well as in other studies.^{2,6,12,20} The *IL28B-CC* genotype is an exception to this pattern. This genotype is associated with a higher frequency of spontaneous HCV clearance in UHS²⁵ and other studies²⁶⁻³⁰ and a higher likelihood of a successful response to Peg-IFN/RBV combination therapy, but paradoxically, it was also associated with higher HCV RNA (among the

European-American participants and UHS subjects overall). A number of previous reports also found the otherwise favorable *IL28B* genotype to be associated with higher baseline HCV RNA,^{4,31,32} (although some other studies did not^{26,27}). The association of *IL28B-CC* genotype with both better response to therapy and higher serum HCV RNA in the absence of treatment seems counterintuitive, but, before therapy, patients with the *IL28B-CC* genotype have lower expression of IFN-stimulated genes induced by the Janus kinase/signal transducers and activators of transcription pathway.^{33,34} Thus, patients with the favorable genotype appear to have less endogenous IFN activity, but greater responsiveness to exogenous IFN- α .

Comparing participants by racial ancestry, African-American UHS participants had the highest HCV RNA levels, despite having the lowest frequency of the *IL28B-CC* genotype. Thus, not only does the lower prevalence of the *IL28B-CC* genotype among African Americans not explain their higher viral loads, but controlling for *IL28B* genotype actually increases the disparity in viral loads between African Americans and both whites and Asian/Amerindian participants. Furthermore, we did not see the association between higher HCV RNA and *IL28B-CC* among the African-American participants. It is possible therefore that additional genetic factors lead to poorer viral control among persons of African ancestry.

Our study has a number of strengths. UHS is a cohort of street-recruited IDUs; therefore, we could compare HCV RNA across ancestral groups or individuals infected with different viral genotypes without the potential biases caused by markedly differing sources of HCV infection or socioeconomic status. Few, if any, of the UHS participants had been treated for HCV infection; therefore, the HCV RNA values among these subjects were not subject to selection by previous HCV treatment. The relatively large size of the cohort provided good statistical power for many comparisons, although our power was low for certain variable categories, including Hispanic or Asian ancestry and viral genotypes 3 or 4. The limitations of our study should be considered as well. The cross-sectional design did not allow us to determine the timing of HCV, HBV, and HIV infections among the participants, and we also could not differentiate the effect of duration of infection (as estimated by number of years of drug injection) from the effect of age because these factors are highly correlated. As mentioned above, we could not determine whether the relationship between duration of infection might represent superinfection, immune senescence, or some other factor that varies

with time or age. Cluster of differentiation (CD)4⁺ lymphocytes counts were not measured for UHS subjects; therefore, we could not consider the extent of immunodeficiency present among the 13.9% of participants in this analysis who were coinfecting with HIV-1. Successful antiretroviral therapy in HIV/HCV-coinfecting individuals may increase HCV RNA levels, at least temporarily, especially among individuals with lower CD4⁺ counts,³⁵ but our data were too limited to allow us to examine this effect.

We performed viral genotyping by direct sequencing, the “gold standard” technique for discriminating HCV types and subtypes.³⁶ This genotyping was based on the NS5B region, which tends to produce more accurate results than the 5'NC region,³⁷⁻³⁹ but this method allowed us to detect only the dominant circulating strain of HCV. An important concern in this analysis is whether methodological differences may account for the discrepancies in HCV RNA levels between different genotypes. We used a third-generation (i.e., bDNA) assay with an analytic sensitivity of 2.5×10^3 copies/mL to measure viral levels. This method amplifies signal, rather than target, which is the basis for classical RT-PCR and transcription-mediated amplification assays. First-generation bDNA assays underestimated levels of HCV genotype 2 and 3,⁴⁰ but third-generation bDNA tests are accurate, reproducible, and well calibrated to the World Health Organization HCV RNA standard.⁴¹ In support of our findings, a previous report of an association between HCV genotype 4 infection and lower HCV RNA levels was based on measurement by PCR and determined that the results were not influenced by viral genotype-specific amplification bias.²⁴

In conclusion, level of HCV viremia, an important predictor of response to HCV treatment, is itself influenced by a wide range of demographic, viral, and host genetic factors. A better understanding of the determinants of HCV viremia might lead to improved treatment of patients with CHC.

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