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Comparison of Functional Variants in *IFNL4* and *IFNL3* for Association with Hepatitis C Virus Clearance

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Abstract

Background & Aims—Genetic polymorphisms within the interferon lambda (IFN- λ) region are strongly associated with hepatitis C virus (HCV) clearance; the *IFNL4*- G/TT (rs368234815) polymorphism, which controls generation of the IFN- λ 4 protein, is more strongly associated with HCV clearance than rs12979860 (the 'IL28B variant'). An *IFNL3* 3' untranslated region polymorphism (rs4803217) has been proposed as a causal variant that may affect HCV clearance by altering *IFNL3* mRNA stability.

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Conflict of Interest: TRO'B and LP-O are inventors on patent applications filed by the National Cancer Institute for the *IFNL4*- G/TT (rs368234815) genotype-based test and the IFN- λ 4 protein. For other authors there are none to declare.

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Methods—We compared *IFNL4*- G/TT and rs4803217 for association with response to pegylated-IFN- α /ribavirin in the VIRAHEP-C and HALT-C trials, and spontaneous HCV clearance in the ALIVE, UHS and WIHS studies. Genotyping was performed with TaqMan assays. We compared differences in mean reduction in HCV RNA levels by genotype and haplotype. For HCV clearance, we calculated p-values comparing c-statistics for *IFNL4*- G/TT and rs4803217 genotypes by a bootstrap approach.

Results—Among European Americans, linkage disequilibrium between *IFNL4*- G/TT and rs4803217 was strong ($r^2=0.89-0.99$) and there were no significant differences between the variants. In African American (AA) individuals enrolled in VIRAHEP-C, HCV RNA at treatment day 28 was more strongly associated with *IFNL4*- G/TT than rs4803217 ($p=0.003$); the *IFNL4*- G:rs4803217-G haplotype, which includes the putatively favorable *IFNL3* allele, was actually associated with the poorest day 28 response ($p=0.03$, comparison to *IFNL4*- G:rs4803217-T haplotype). Among AA participants, associations were stronger for *IFNL4*- G/TT than rs4803217 for undetectable HCV RNA at week 24 in Virahep C ($p=0.03$) and week 20 in HALT-C ($p=0.03$), as well as for spontaneous HCV clearance ($p=0.048$).

Conclusion—*IFNL4*- G/TT is the primary IFN- λ region polymorphism for impaired HCV clearance.

Keywords

genetics; IL28B; *IFNL3*; *IFNL4*; innate immunity; interferon lambda; treatment; viral clearance

Background

Impaired clearance of hepatitis C virus (HCV) is strongly associated with certain genetic polymorphisms in the interferon lambda (IFN- λ) region. The four genes in this chromosomal locus were discovered relatively recently. In 2003, *IFNL1*, *IFNL2* and *IFNL3* (previously, *IL29*, *IL28A* and *IL28B*, respectively) were identified by two independent research groups through genome-based computational prediction [1–3]; proteins encoded by these genes are highly similar to each other with 96% amino acid identity between IFN- λ 2 and IFN- λ 3 [1, 2]. *IFNL4* was discovered in 2013 through RNA sequencing of primary human hepatocytes that had been stimulated to mimic HCV infection [4].

Prior to the discovery of *IFNL4*, a genome-wide association study (GWAS) found single nucleotide polymorphism (SNP) rs12979860 to be the variant most strongly associated with response to treatment with pegylated-IFN- α plus ribavirin for chronic hepatitis C [5]. A number of contemporaneous studies reported consistent findings for both treatment response and spontaneous clearance of HCV [6–9]. Although commonly referred to as an ‘IL28B’ or *IFNL3* variant, rs12979860 actually lies in intron 1 of *IFNL4* and, therefore, is properly termed *IFNL4*-rs12979860 [4]. The *IFNL4*- G/TT frame-shift variant (rs368234815, originally designated ss469415590) lies in exon 1 of *IFNL4*. The *IFNL4*- G allele creates an open reading frame for the IFN- λ 4 protein, whereas the alternative *IFNL4*-TT allele eliminates the IFN- λ 4 protein [4]. *IFNL4*- G is in strong linkage disequilibrium (LD) with the unfavorable rs12979860-T allele, however, *IFNL4*- G is associated with impaired HCV clearance more strongly than rs12979860-T [4, 10, 11], especially in individuals of African

ancestry, in whom LD between the two alleles is weakest [4]. Given that *IFNL4*- G/TT shows the strongest association for genetic differences in viral clearance and that the *IFNL4*- G allele controls generation of IFN- λ 4, *IFNL4*- G/TT is a very plausible candidate as the causal variant underlying observed genetic associations with HCV clearance.

SNP rs4803217, located in the 3' untranslated region (UTR) of *IFNL3*, has also been proposed as a causal variant for HCV clearance [12]. Variants in 3' UTRs can affect binding of miRNAs and regulation of gene expression [13, 14], and rs4803217-G reportedly decreases degradation of *IFNL3* mRNA upon HCV infection through this mechanism [12]. It was proposed, therefore, that rs4803217-G could be associated with enhanced HCV clearance, however, data on rs4803217 genotype and HCV clearance are limited.

In vitro studies of HCV clearance can be useful for identifying genetic variants that may be functional in humans, however, genetic association studies are needed to determine which variant is more strongly associated with outcome and, therefore, more likely to be causal. Many genetic variants in the IFN- λ region are in strong LD and, for such highly linked variants, it is important to examine the effect in multiple populations, especially populations of African ancestry in which LD tends to be weaker. To address whether *IFNL4*- G/TT or rs4803217 is the primary functional variant in the IFN- λ region, we compared genetic associations of these polymorphisms for response to treatment of chronic hepatitis C and spontaneous HCV clearance among African American (AA) and European American (EA) individuals.

Methods

Subjects

As previously described, [4] we examined genetic associations in two studies of response to treatment with pegylated IFN- α plus ribavirin that were sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases. The Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C (VIRAHEP-C) was designed to compare response to treatment in AA patients with chronic hepatitis C to otherwise similar patients of European ancestry [15]. In VIRAHEP-C, patients with HCV genotype 1 infection who had not undergone previous treatment for chronic hepatitis C received a standard regimen of pegylated IFN- α -2a (180 μ g/week) plus ribavirin (1000–1200 mg/day) for up to 48 weeks. Study end points included the following: decrease in HCV RNA levels between baseline and various treatment time points; week 24 response (absence of detectable HCV RNA in serum after 24 weeks of therapy); end-of-treatment response (absence of HCV RNA after 48 weeks of therapy); and sustained virological response (SVR; absence of HCV RNA 24 weeks after treatment was stopped). The Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial was a study of patients with advanced chronic hepatitis C who had failed previous interferon-based treatment. [16, 17] Patients with other liver diseases, human immunodeficiency virus (HIV) infection, active illicit drug use or current alcohol abuse were excluded. During the lead-in phase of HALT-C, patients underwent retreatment with pegylated-IFN- α -2a (180 μ g/week) plus ribavirin (1000–1200 mg/day). Subjects with undetectable HCV RNA at week 20 remained on combination treatment through week 48 and were followed until week 72. Subjects with undetectable HCV RNA at weeks 48 and 72

were considered to have an SVR. Investigations of genetic associations in VIRAHEP-C and HALT-C were conducted in those participants who provided written consent for genetic testing. VIRAHEP-C and HALT-C protocols were approved by the Institutional Review Boards (IRBs) of the participating institutions.

Analyses of genetic associations with spontaneous clearance of HCV infection were performed in three studies, as described in previous publications [4, 18]. The AIDS Linked to Intravenous Experience (ALIVE) cohort is an ongoing study that has followed injection drug users (IDUs) in Baltimore since 1988 [19]. HCV infection was established by detection of HCV antibody (anti-HCV) by enzyme immunoassay (EIA) and recombinant immunoblot assay (RIBA [version 3.0]; Novartis). Individuals with cleared HCV infection had anti-HCV (as confirmed by RIBA) and undetectable HCV RNA in serum or plasma without having received any HCV therapy. Written informed consent for genetic testing was obtained from all participants. The study was approved by the IRB at Johns Hopkins Bloomberg School of Public Health.

As previously described, Urban Health Study (UHS) investigators recruited IDUs from street settings in six inner-city San Francisco Bay area neighborhoods from 1986 through 2002, drawing serial cross-sectional samples every six months [20]. Individuals 18 years of age or older were eligible for enrollment if they had injected drugs within the past 30 days or previously enrolled in the UHS study. New participants were screened for visible signs of recent or chronic injection (i.e., venipuncture sites or scars). Interviews were conducted using standardized questionnaires and blood samples were collected from participants who provided written informed consent. The present study included unduplicated IDUs recruited between 1998 and 2000 [21]. Participants who were positive for HCV antibody were divided into two groups based on their HCV RNA result: 'chronic' (positive for HCV RNA) or 'cleared' (negative for HCV RNA and positive for antibody). All subjects with cleared infection were included in the study and frequency matched to those with chronic infection on the basis of self-reported ethnicity and age. Study procedures were approved by an IRB of the National Cancer Institute and the Committee on Human Subjects Research at the University of California, San Francisco.

The Women's Interagency HIV Study (WIHS) is a prospective cohort study of HIV-seropositive and at-risk HIV-seronegative women who were enrolled at six clinical sites [22]. Initial enrollment was conducted during 1994–1995, a second recruitment occurred during 2001–02, and a third recruitment period occurred in 2011. Subjects are followed semi-annually with physical exams, specimen collection including blood, and detailed questionnaires regarding health and behavior. The WIHS protocol was approved by each local IRB, and all participants included in this analysis provided written informed consent for genetic testing. The present study focused on the women who were anti-HCV seropositive at the enrollment visit and provided information on race/ethnicity.

Laboratory

Genotyping of *IFNL4*- G/TT (rs368234815) was performed previously in the Laboratory of Translational Genomics, NCI with a custom TaqMan allelic discrimination genotyping assay (Life Technologies, Foster City, California) [4, 18]. For the present study, genotyping of

IFNL3-rs4803217 was performed in the same laboratory with a custom TaqMan assay (Life Technologies, Foster City, California) as previously described [23]. Genotyping was performed by staff who had no knowledge of the clinical phenotype or racial ancestry of the study subjects. For WIHS and UHS, duplicate samples were genotyped for rs4803217. The panel of blinded specimens from WIHS included 43 samples that were tested in duplicate for rs4803217 with 95.3% concordance. For UHS, 130 specimens were tested in duplicate for rs4803217 and 99.2% of the pairs produced concordant results.

In VIRALHEP-C, serum HCV RNA concentrations on day 0, 1, 2, 7, 14, and 28 had been measured with the COBAS Amplicor Hepatitis C Virus Monitor Test, 2.0™ assay (sensitivity 600 international units (IU)/ml; Roche Molecular Diagnostics, Alameda, CA) [24]. In HALT-C, serum samples that had undetectable HCV RNA by that assay were also tested using the qualitative Roche COBAS Amplicor HCV Test, v. 2.0 assay (lower limit of detection, 100 IU/mL; Roche Molecular Diagnostics, Alameda, CA).

Statistical Analysis

We examined LD of *IFNL3*-rs4803217 with *IFNL4*- G/TT and with rs12979860 by calculating the correlation coefficient (r^2) using Haploview software (version 4.2) [25].

Statistical comparisons of *IFNL4*- G/TT and rs4803217 were limited to subjects whose DNA specimen was successfully genotyped for both variants. Analyses were stratified by race (self-reported) to control potential confounding. We used the Kruskal-Wallis test to compare median HCV RNA levels between genotypes for each variant (e.g., *IFNL4*-TT/TT versus *IFNL4*- G/ G). As previously described [4], we compared mean HCV RNA levels in each of the three *IFNL4*- G/TT genotype groups (i.e., G/ G, G/TT, TT/TT) with the respective rs4803217 genotype groups (TT, GT, GG). To determine global statistical significance of these three mean differences, we computed the covariance matrix of the mean differences using a bootstrap procedure. We re-sampled individuals in the study with replacement, and then computed the three differences of the mean RNA levels in the three genotype groups in this bootstrap dataset. We repeated this calculation 10,000 times and used the bootstrap replicates to compute the covariance matrix of the mean differences of the original sample. This covariance matrix was used to compute a three degrees-of-freedom Wald statistic to test the null hypothesis that there was no difference in mean HCV RNA decreases for *IFNL4*- G/TT and rs4803217.

For dichotomous outcomes (e.g., SVR, spontaneous clearance) we calculated the odds ratio (OR) and accompanying Wald chi-square p-value (Proc Logistic, SAS, Cary, NC) in logistic models that were adjusted for pre-treatment HCV RNA level and fibrosis stage. We examined both general and additive genetic models. We determined the area under the receiver operating characteristic curve (AUROC or c-statistic) [26] for genotypes of each variant for each outcome and computed a p-value for differences in AUROC values (i.e., c-statistic) based on a chi-square test (1 df) that used a bootstrap variance estimate computed by resampling subjects with replacement and repeating the AUROC computations for each bootstrap sample. Haplotype analyses were conducted using the function *haplo.glm* from the package *haplo.stats* in R.

Results

Linkage Disequilibrium

We examined LD between the rs4803217, *IFNL4*- G/TT and rs12979860 variants in the AA and EA participants in this study (Supplementary Figure 1). LD between the *IFNL4*- G and rs4803217-T alleles was weaker in the AA participants ($r^2=0.77$) than in the EA subjects ($r^2=0.90$), whereas, LD between the rs4803217 and rs12979860 SNPs was the same ($r^2=0.92$) in the AA and EA subjects.

African American Participants

Treatment Response—AA participants in VIRAHEP-C (n=170) and HALT-C (n=142) are described in Table 1. Patients in the two studies were similar with regard to age (medians, 49 and 51 years for VIRAHEP-C and HALT-C, respectively), HCV genotype (all or virtually all genotype 1) and serum HCV RNA levels (medians, 6.4 log₁₀ IU/mL); most participants in both studies were male. By design, HALT-C enrolled patients with more advanced hepatic fibrosis such that 89.4% had an Ishak fibrosis score >3, compared to 33.5% in VIRAHEP-C. Frequencies of the unfavorable *IFNL4*- G allele and of rs4803217-T were higher in HALT-C than VIRAHEP-C, reflecting that, by design, HALT-C participants had failed previous treatment with IFN- α , whereas VIRAHEP-C participants were treatment naive. LD between *IFNL4*- G and rs4803217-T was similar in VIRAHEP-C ($r^2=0.79$) and HALT-C ($r^2=0.74$).

In VIRAHEP-C, the decrease in HCV RNA during the first 28 days of treatment was associated more strongly with *IFNL4*- G/TT than rs4803217 ($p=0.003$, difference in mean values; Figure 1). For example, at treatment day 28, the viral decline for the most favorable *IFNL4* genotype (TT/TT) was 2.84 log₁₀ IU/ml compared to 2.58 log₁₀ IU/ml for the corresponding rs4803217-G/G genotype and, for the unfavorable genotypes, the decline was 0.87 log₁₀ IU/ml for *IFNL4*- G/ G and 1.02 log₁₀ IU/ml for rs4803217-T/T (Supplementary Table 1).

To examine whether rs4803217 might modify the effect of *IFNL4*- G/TT, we examined compound genotypes and haplotypes based on both variants. In contrast to the hypothesis that the rs4803217-G allele might affect viral clearance favorably, the 15 VIRAHEP-C patients with the *IFNL4*- G/ G: rs4803217-T/G compound genotype had the least decline in HCV RNA at day 28 of treatment (0.62 log₁₀ IU/ml; $p=0.03$ compared to patients with the *IFNL4*- G/ G: rs4803217-T/T genotype [Table 2]). HCV RNA decline was also lower in individuals with the *IFNL4*- G/TT: rs4803217-G/G genotype compared to those with the *IFNL4*- G/TT: rs4803217-T/G genotype, although that difference was not statistically significant ($p=0.08$). A haplotype analysis of the decrease in HCV RNA levels at day 28 of treatment yielded similar results (Supplementary Table 2). As expected, the *IFNL4*-TT: rs4803217-G haplotype was associated with a much greater decrease in HCV RNA than the *IFNL4*- G: rs4803217-T haplotype (coefficient, 0.73; $p=0.0001$). The haplotype that paired the unfavorable *IFNL4*- G allele with the putatively favorable rs4803217-G allele was associated with a smaller decline in HCV RNA than *IFNL4*- G: rs4803217-T (coefficient, -0.50; $p=0.03$).

We also compared *IFNL4*- G/TT and rs4803217 for associations with undetectable HCV RNA at several treatment time points in VIRAHEP-C and in HALT-C (Table 3). In VIRAHEP-C, the association was significantly stronger for *IFNL4*- G/TT than rs4803217 at treatment week 24 (p=0.03, difference in AUROC; Supplementary Table 3), at end of treatment (p=0.02) and for SVR (p=0.03). A similar pattern was seen for treatment response in the HALT-C trial (Table 3), although only the finding at week 20, reached statistical significance (p=0.03, difference in AUROC; Supplementary Table 3). In haplotype analyses for the three treatment time points in VIRAHEP-C and in HALT-C (Supplementary Table 4), associations with response were weaker for *IFNL4*- G: rs4803217-G than *IFNL4*- G: rs4803217-T, but none of the associations were statistically significant. The corresponding compound genotypes analyses lacked statistical power (data not shown).

Spontaneous Clearance—Characteristics of participants in ALIVE, UHS and WIHS are presented in Table 4. Those enrolled in UHS were somewhat older (median, 46 years) compared to those enrolled in the other two studies (medians, ~40 years). By design, all WIHS participants were female (most participants in ALIVE and UHS were male) and all participants in ALIVE and UHS had a history of injection drug use (WIHS, 81.5%). The prevalence of HIV-1 infection ranged from 85.9% in WIHS, which selectively enrolled HIV-1-infected women, to 12.4% in UHS. The r^2 values for LD between *IFNL4*- G and rs4803217-T were: ALIVE, 0.75; UHS, 0.77; WIHS, 0.80.

Table 5 shows associations for *IFNL4*- G/TT and rs4803217 with spontaneous clearance in the three individual studies and pooled results. In the combined analysis of 1,475 AA participants, comparison of *IFNL4*-TT/TT to *IFNL4*- G/ G yielded an OR of 4.14, whereas the OR for the corresponding comparison of rs4803217 genotypes was 3.22. Overall, the AUROC was 0.63 for the three *IFNL4*- G/TT genotypes and 0.61 for the rs4803217 genotypes (p=0.048, difference in AUROC). Results from each individual study were generally consistent with the pooled analysis (Table 5).

In contrast to the haplotype analysis of treatment response, in which *IFNL4*- G: rs4803217-G was less favorable than *IFNL4*- G: rs4803217-T, we saw no difference between these haplotypes for spontaneous HCV clearance (OR, 0.97; 95% CI, 0.56–1.67; p=0.90; Supplementary Table 5). Similarly, the pooled compound genotype analysis for spontaneous viral clearance showed no difference between the *IFNL4*- G/ G: rs4803217-G/G genotype and the *IFNL4*- G/ G: rs4803217-T/G genotype (OR, 0.96; p=0.93; Supplementary Table 6). Consistent with our finding that rs4803217-G actually appeared to be unfavorable for treatment response in the presence of *IFNL4*- G, individuals with the *IFNL4*- G/TT: rs4803217-T/T genotype were more likely to have cleared HCV than those with *IFNL4*- G/TT: rs4803217-T/G genotype (OR, 2.87), however, that finding was of borderline statistical significance (p=0.06) and there was no difference in clearance between those with the *IFNL4*- G/TT: rs4803217-G/G and *IFNL4*- G/TT: rs4803217-T/G genotypes (OR, 0.92; p=0.85).

European American Participants

We also examined associations for *IFNL4*- G/TT and rs4803217 genotypes among European American participants, however, strong LD between the variants limited the statistical power for those comparisons. Among the 183 European American patients enrolled in VIRALHEP-C, LD was nearly complete ($r^2 = 0.99$) and, therefore, genotype comparisons were not informative (data not shown). For HALT-C (n=743), r^2 between *IFNL4*- G and rs4803217-T was 0.89; *IFNL4*- G/TT was somewhat more strongly associated with treatment response than rs4803217 (Supplementary Tables 7 and 8). Similarly, with regard to spontaneous HCV clearance among the European American participants, r^2 values were 0.90 and 0.89 in UHS and WIHS, respectively, and the association with spontaneous clearance was somewhat stronger for *IFNL4*- G/TT than rs4803217 (Supplementary Tables 9 and 10).

Discussion

In vitro studies demonstrated that the rs4803217-G allele within the 3'UTR of *IFNL3* decreased HCV-induced degradation of *IFNL3* mRNA and, on that basis, rs4803217 was proposed as a causal variant that improves HCV clearance [12]. In the present study, we compared rs4803217 to *IFNL4*- G/TT, a functional variant that controls generation of the IFN- λ 4 protein. Among participants of African ancestry, associations with HCV clearance (response to treatment of chronic hepatitis C with pegylated IFN- α plus ribavirin and spontaneous HCV clearance) were consistently stronger for *IFNL4*- G/TT than rs4803217. LD between these variants was high in subjects of European ancestry and we observed no statistically significant differences between *IFNL4*- G/TT and rs4803217 in that population. Based on information from the 1000 Genomes project, LD between *IFNL4*- G/TT and rs4803217 was complete in Asians ($r^2=1.0$), which indicates these variants will provide equal predictive value in patients of Asian ancestry. These results provide additional evidence that the *IFNL4*- G/TT variant is the best genetic predictor of HCV clearance.

This study provides no indication that the rs4803217-G allele improves HCV clearance. In fact, it offers some evidence that rs4803217-G may have a negative effect on HCV clearance, since the haplotype consisting of *IFNL4*- G and rs4803217-G was associated with even poorer HCV RNA decline at day 28 of treatment than the *IFNL4*- G:rs4803217-T haplotype. We could not confirm an unfavorable association for the *IFNL4*- G:rs4803217-G haplotype in the analysis of spontaneous clearance, however, our study provided no evidence that rs4803217-G increases HCV clearance.

To our knowledge, this is the first study comparing *IFNL4*- G/TT and rs4803217 for HCV clearance. Two smaller studies compared rs4803217 to rs12979860, a variant that is in strong LD with rs4803217 and which is associated with HCV clearance less strongly than *IFNL4*- G/TT [4, 10]. Among 197 HCV/HIV-1 co-infected patients from Barcelona, associations for response to treatment with pegylated IFN- α plus ribavirin were comparable for rs4803217 and rs12979860 genotypes [27]. Those findings are consistent with the data from EA individuals in our study. An analysis of spontaneous HCV clearance in an Egyptian population (n=261) suggested a slightly weaker genotype association for rs4803217 than rs12979860 [28].

The available evidence strongly supports the *IFNL4*- G/TT polymorphism and generation of the IFN- λ 4 protein as the explanation for observed associations of IFN- λ region variants with HCV clearance. As demonstrated here and previously [4, 10, 11, 18], *IFNL4*- G/TT is the best variant for predicting HCV clearance across a range of populations. Furthermore, this polymorphism is highly functional – the *IFNL4*- G allele generates the IFN- λ 4 protein, while the frameshift caused by the *IFNL4*-TT allele abrogates that protein [4]. Furthermore, structural variation in IFN- λ 4 appears to affect HCV clearance. A non-synonymous *IFNL4* polymorphism (rs117648444-T/C, Pro70Ser) that exists only on a haplotype with the *IFNL4*- G allele generates an alternative form of IFN- λ 4 protein [4]. Individuals with the IFN- λ 4-S70 variant have rates of treatment response and spontaneous HCV clearance that are intermediate between those for patients with IFN- λ 4-P70 and those who generate neither form of IFN- λ 4 (i.e., those with the *IFNL4*-TT/TT genotype) [29, 30], which is consistent with the observation that the IFN- λ 4-S70 protein is less biologically active than IFN- λ 4-P70 [29]. In addition, the *IFNL4*-TT allele explains the signature of positive selection that has been observed in the IFN- λ chromosomal region (and evidence of selection is stronger for *IFNL4*- G/TT than rs4803217)[31, 32]. Selection at this locus resulted in *IFNL4*-TT being the major form in Asia, while *IFNL4*- G is the major allele in Africa [4]; *IFNL4*- G/TT is among the top 0.5% of polymorphisms with regard to genome-wide differentiation between African and East Asian populations [31]. Selection for *IFNL4*-TT may not have been driven by HCV infection [33], however, the data are consistent with a favorable role for *IFNL4*-TT in viral infection.

This study was not designed to elucidate how the IFN- λ 4 protein might impair HCV clearance and that functional mechanism remains unclear [33]. Interferons are generally considered antiviral cytokines and there is evidence that IFN- λ 4 has anti-viral activity [4, 34], yet genetic association studies link generation of IFN- λ 4 to impaired HCV clearance. Although IFN- λ 4 most closely resembles IFN- λ 3, these proteins share only 29% amino acid identity [4]. Like other members of the IFN- λ family, IFN- λ 4 signals through the IFN- λ receptor complex, induces expression of interferon-stimulated genes via the JAK-STAT signaling pathway and demonstrates anti-viral activity [4, 34], yet, in contrast to IFN- λ 1-3, IFN- λ 4 is only weakly secreted [4, 34]. Additional work is needed to resolve the apparent paradox of IFN- λ 4 and HCV clearance.

Regimens based on direct-acting antiviral agents (DAA) are becoming the standard of care for HCV infection, although the cost of these treatments may limit their wide-scale implementation [35]. In some trials, very high response rates have made it difficult to determine if genotype for *IFNL4* variants can impact response to DAA regimens [36], however, results from several other studies have shown that this is the case [36–39]. The fixed-dose combination of ledipasvir and sofosbuvir has recently become available for treatment of HCV infection. In the phase III ION-3 trial [40], 423 previously untreated HCV genotype 1-infected patients without cirrhosis had outcome data after receiving ledipasvir/sofosbuvir for 8 or 12 weeks. The published subgroup analysis found no significant differences in SVR rates by rs12979860 genotype, however, enrollees with missing outcome data constituted a large proportion of those counted as treatment failures [40]. In an ‘efficacy analysis’ of subgroup differences in treatment based on the published data [39], we found that SVR rates

varied significantly by rs12979860 genotype and exceeded 98% in individuals with the rs12979860-CC genotype (which is very strongly linked with the *IFNL4*-TT/TT genotype) who were treated for 8 weeks. Genotype for rs12979860 remained informative even after HCV RNA was considered. [41] Current treatment guidelines either do not endorse ledipasvir/sofosbuvir regimen for <12 weeks [42] or do so only with caution. [43] However, the results from the efficacy analysis suggest that treatment-naïve patients with HCV genotype 1 and the *IFNL4*-TT/TT genotype are highly likely to respond to 8 weeks of ledipasvir/sofosbuvir. Thus, host genotype may be useful for personalizing treatment with DAA regimens in order to decrease treatment costs. For that purpose, *IFNL4*- G/TT, possibly in combination with the *IFNL4* Pro70Ser polymorphism, would be the most predictive variant across a range of populations.

A major strength of this study is its inclusion of AA participants in whom LD between *IFNL4*- G/TT and rs4803217 is much weaker than in Europeans or Asians. Among AA subjects, we saw statistically significant differences between these variants in genotype associations for HCV clearance, whereas there were no significant differences between the genotypes in EA individuals. A limitation of the study is the low statistical power for some analyses. Results for comparisons of *IFNL4*- G/TT and rs4803217 were consistent, but results for HALT-C failed to achieve statistical significance. Furthermore, although the study provides strong evidence against the hypothesis that rs4803217-G increases HCV clearance [12], our efforts to determine if there was an association in the opposite direction (i.e., that rs4803217-G is associated with impaired viral clearance) were limited by low statistical power.

In conclusion, this study provides additional evidence that the *IFNL4*- G allele, which generates the IFN-λ4 protein, is the primary variant for impaired clearance of HCV. In the clinical setting, genotype for *IFNL4*- G/TT will provide the best genetic prediction for HCV clearance. Future studies should examine how IFN-λ4 interferes with viral clearance and whether *IFNL4*- G/TT plays a role in other viral infections.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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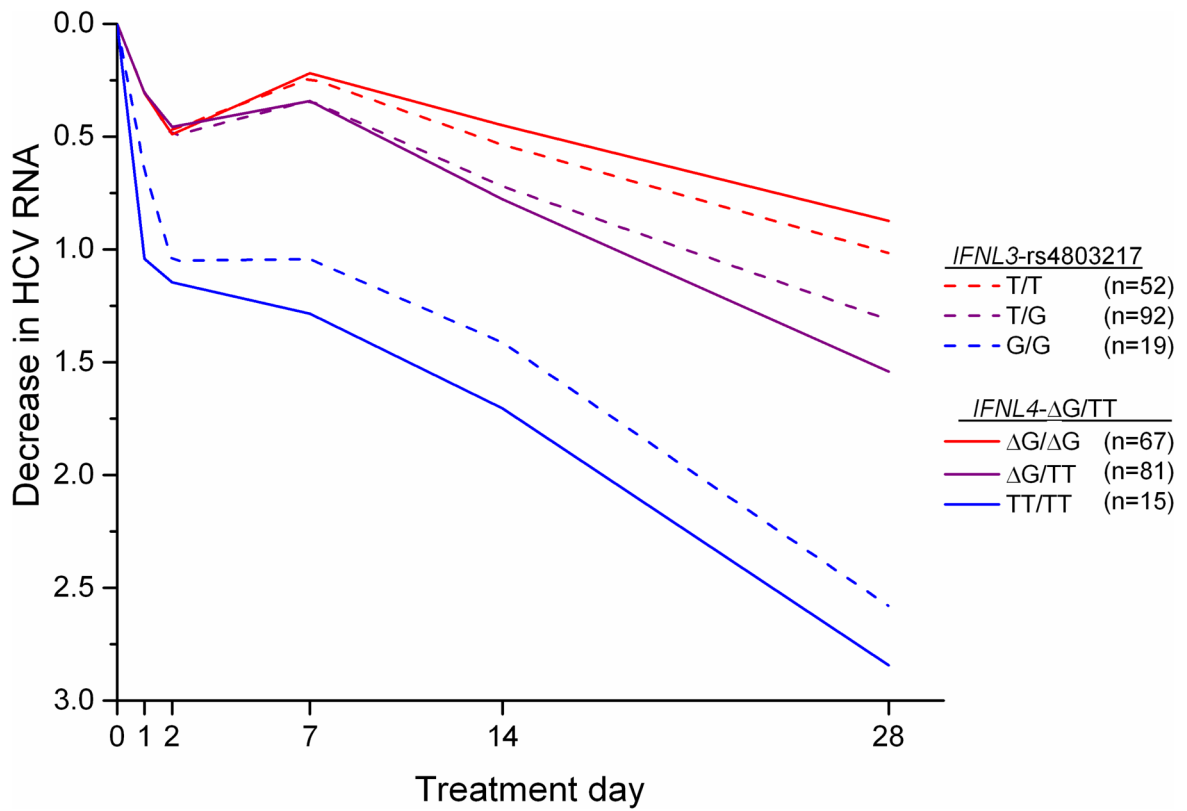


Figure 1.

Median decrease in HCV RNA levels (\log_{10} international units (IU)/ml) in African American participants in the VIRAHEP-C study during the first 28 days of treatment with pegylated-interferon- α plus ribavirin. $P = 0.003$ for the mean differences in HCV RNA levels at day 28 for each of the three *IFNL4*- G/TT (rs368234815) genotype groups relative to the respective *IFNL3* rs4803217 genotype groups.

Table 1

Characteristics of African American participants in the VIRAHEP-C and HALT-C trials of response to treatment with pegylated-interferon- α plus ribavirin

Characteristic	VIRAHEP-C (n = 170)		HALT-C (n = 142)	
Age (Median, IQR)	49.1	45.6 – 52.8	51	46.0 – 56.0
Male (n, %)	111	65.3	81	57.0
HCV Genotype 1 (n, %)	170	100.0	137	96.5
HCV RNA level (log ₁₀ IU) (median, IQR)	6.4	5.6 – 6.7	6.4	6.1 – 6.8
Ishak Fibrosis Score				
Missing (n, %)	1	0.6		
0–2 (n, %)	112	65.9	15	10.6
3–4 (n, %)	49	28.8	81	57.0
5–6 (n, %)	8	4.7	46	32.4
<i>IFNL4</i> - G allele frequency (%)		66.2		69.7
<i>IFNL3</i> rs4803217-T allele frequency (%)		60.6		64.8
Linkage disequilibrium (r ²)		0.79		0.74

IQR, Interquartile range

IU, International units

Table 2

Median decrease in HCV RNA levels (\log_{10} international units (IU)/ml) in African American participants in the VIRAHEP-C study at day 28 of treatment with pegylated-interferon- α plus ribavirin, by compound genotype for *IFNL4*- G/TT (rs368234815) and *IFNL3* rs4803217.

<i>IFNL4</i> - G	rs4803217	N	HCV RNA	P-value
G/ G	T/T	52	1.02	
G/ G	T/G	15	0.62	0.03*
G/TT	T/G	77	1.58	
G/TT	G/G	4	0.82	0.08**
TT/TT	G/G	15	2.84	

* Compares patients with the *IFNL4*- G/ G: rs4803217-T/G genotype to those with the *IFNL4*- G/ G: rs4803217-T/T genotype

** Compares patients with the *IFNL4*- G/TT: rs4803217-G/G genotype to those with the *IFNL4*- G/TT: rs4803217-T/G genotype

Comparison of *IFNL4*- G/TT (rs368234815) and *IFNL3* rs4803217 for genotype associations among African-American participants for association with response to treatment with pegylated interferon-alfa plus ribavirin, VIRAHEP-C and HALT-C Trial.

Table 3

Variant	Genotype	N	Treatment Time Point								
			Week 20/24*			End-of-Treatment			SVR		
			Response Rate (%)	aOR	p-value	Response Rate (%)	aOR	p-value		Response Rate (%)	aOR
VIRAHEP-C (n = 170)**											
<i>IFNL4</i>- G/TT	G/ G	71	35.2	Ref.		32.4	Ref.	21.1	Ref.		
	G/TT	83	55.4	2.81	0.004	44.6	1.88	0.07	31.3	2.01	0.08
	TT/TT	16	68.8	5.10	0.01	56.3	2.93	0.07	43.8	3.28	0.05
	Additive			2.48	0.001		1.77	0.03		1.87	0.03
rs4803217	T/T	56	39.3	Ref.		35.7	Ref.	25.0	Ref.		
	T/G	94	51.1	2.01	0.06	41.5	1.53	0.25	28.7	1.53	0.3
	G/G	20	60.0	3.02	0.05	50.0	2.12	0.17	35.0	2.01	0.24
	Additive			1.81	0.03		1.47	0.14		1.44	0.20
HALT-C Trial (n = 142)											
<i>IFNL4</i>- G/TT	G/ G	64	9.4	Ref.		7.8	Ref.	3.1	Ref.		
	G/TT	70	18.6	3.38	0.047	14.3	2.70	0.13	8.6	4.41	0.13
	TT/TT	8	50.0	22.74	0.002	37.5	13.58	0.01	25.0	24.24	0.02
	Additive			4.29	0.002		3.38	0.01		4.86	0.02
rs4803217	T/T	59	10.2	Ref.		8.5	Ref.	3.4	Ref.		
	T/G	66	18.2	3.12	0.07	13.6	2.56	0.16	7.6	4.32	0.14
	G/G	17	29.4	6.43	0.03	23.5	5.04	0.05	17.6	10.66	0.04
	Additive			2.59	0.01		2.27	0.04		3.21	0.03

* In VIRAHEP-C response is at 24 weeks after initiation of treatment; in HALT-C response is at 20 weeks after initiation of treatment

** One subject is lost from the adjusted analyses for VIRAHEP-C due to missing data for fibrosis

SVR, sustained virological response; aOR, odds ratio adjusted for pre-treatment HCV RNA level and fibrosis stage

Characteristics of African American participants in the studies of spontaneous HCV clearance: ALIVE, UHS, WIHS.

Table 4

	ALIVE (n = 593)		UHS (n = 363)		WIHS (n = 519)	
Age, median (IQR)	40.3	35.9 – 44.5	46	42.0 – 50.0	40.7	37.2 – 44.3
Male (%)	443	74.7	227	62.5	0	0.0
Injection drug use [ever] (%)	593	100.0	363	100.0	423	81.5
HIV-1-infected (%)	318	53.6	45	12.4	446	85.9
<i>IFNL4</i> - G allele frequency (%)		63.9		58.3		61.8
<i>IFNL3</i> rs4803217-T allele frequency (%)		60.6		56.2		57.2
Linkage disequilibrium(r^2)		0.75		0.77		0.80

IQR, Interquartile range

Table 5

African American participants from ALIVE, UHS and WIHS with chronic or cleared HCV infection, genotype proportions for *IFNL4*- G/TT (rs368234815) and *IFNL3* rs4803217 variants, and corresponding Mantel-Haenszel (M-H) odds ratios, 95% confidence intervals (95% CI) and p-values for each association.

Variant	<i>IFNL4</i> - G/TT						rs4803217
	Genotype	G/ G	G/TT	TT/TT	T/T	T/G	
ALIVE	Chronic (%) [N= 534]	43.3 [231]	45.9 [245]	10.9 [58]	40.6 [217]	44.8 [239]	14.6 [78]
	Cleared (%) [N= 59]	23.7 [14]	39.0 [23]	37.3 [22]	23.7 [14]	30.5 [18]	45.8 [27]
	Odds ratio	Ref.	1.55	6.26	Ref.	1.17	5.37
	95% CI	-	0.78–3.08	3.02–12.98	-	0.57–2.45	2.68–10.76
UHS	p-value		0.21	8.3×10 ⁻⁷		0.67	2.2×10 ⁻⁶
	Chronic (%) [N=276]	36.6 [101]	50.0 [138]	13.4 [37]	32.2 [89]	52.5 [145]	15.2 [42]
	Cleared (%) [N=87]	24.1 [21]	47.1 [41]	28.7 [25]	25.3 [22]	47.1 [41]	27.6 [24]
	Odds ratio	Ref.	1.43	3.25	Ref.	1.14	2.31
WIHS	95% CI		0.80–2.57	1.63–6.49		0.64–2.05	1.17–4.59
	p-value		0.23	8.4×10 ⁻⁴		0.65	0.02
	Chronic (%) [N=437]	41.2 [180]	46.5 [203]	12.4 [54]	36.2 [158]	46.5 [203]	17.4 [76]
	Cleared (%) [N=82]	31.7 [26]	32.9 [27]	35.4 [29]	26.8 [22]	37.8 [31]	35.4 [29]
TOTAL	Odds ratio	Ref.	0.92	3.72	Ref.	1.10	2.74
	95% CI	-	0.52–1.65	2.02–6.85	-	0.61–1.97	1.48–5.08
	p-value		0.78	2.0×10 ⁻⁵		0.76	0.001
	Chronic (%) [N=1,247]						
Cleared (%) [N=228]	M-H Odds ratio	Ref.	1.25	4.14	Ref.	1.15	3.22
	95% CI		0.88–1.77	2.81–6.11		0.80–1.64	2.20–4.72
	p-value		0.21	7.2×10 ⁻¹³		0.45	2.0×10 ⁻⁹