BRIEF REPORT

IFNL4-ΔG Genotype Is Associated With Slower Viral Clearance in Hepatitis C, Genotype-1 Patients Treated With Sofosbuvir and Ribavirin

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Response to pegylated interferon-alpha and ribavirin (IFN-α/RBV) treatment for chronic hepatitis C virus (HCV) infection is influenced by host genetic factors, but their role for IFN-α–free, direct-acting antiviral (DAA) regimens is unclear. An exonic deletion allele (*IFNL4*-ΔG) bolsters the established association with IFN-α/RBV therapy treatment outcome of another *IFNL4* variant, rs12979860, which is located upstream of *IFNL3* (*IL28B*). We report that in patients treated with the DAA sofosbuvir along with RBV, *IFNL4*-ΔG is associated with slower early viral decay, due to slower loss of free virus ($P = .039$) and decreased drug efficacy ($P = .048$), suggesting functional relevance of IFN-λ4 in IFN-α–free DAA therapies.

Keywords. viral kinetics; pharmacokinetics; IL28B; IFNL4; haplotype; SVR; hepatitis C virus; DAA therapy; relapse.

Received 30 September 2013; accepted 11 December 2013; electronically published 23 December 2013.

Presented in part: The International Liver Congress. Amsterdam, the Netherlands, 24–28 April 2013. Late-breaker poster.

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The Journal of Infectious Diseases 2014;209:1700–4

DOI: 10.1093/infdis/jit827

In treatment-naive hepatitis C virus (HCV) genotype-1 patients, the current standard-of-care treatment, an NS3 protease inhibitor with IFN-α/RBV, results in sustained virological response (SVR) rates of 63%–75% [1]. However, many patients remain untreated due to relative or absolute contraindications, fear of adverse events, or pill burden. Treatment of chronic HCV infection is evolving towards IFN-α–free, direct-acting antiviral (DAA) regimens that specifically target the HCV protease (NS3/4A), RNA polymerase (NS5B), or nonstructural protein NS5A. While phase 2 and 3 studies of DAA therapies demonstrate promising efficacy and tolerability [2], issues of cost and access to treatment will remain relevant. Identification of host factors that affect differential response to DAA therapies could permit personalization of HCV clinical management.

Previously, the strongest known pretreatment host predictor of response to IFN-α/RBV therapy for HCV genotype-1 infection was the genotype of a single nucleotide polymorphism (SNP) rs12979860 (C/T), commonly referred to as the "*IL28B*" genotype (reviewed in [3]). Homozygosity for the rs12979860-C allele (*IL28B*-CC genotype) is associated with faster viral kinetic (VK) decline and higher odds of achieving SVR with IFN-α/RBV therapy (reviewed in [3]). Recently, it was shown that rs12979860 is located within the first intron of the novel human IFN-lambda-4 (*IFNL4*) gene and 367 base pairs from a functional dinucleotide variant (rs368234815, previously designated as ss469415590; *IFNL4*-TT/ΔG) located within the first *IFNL4* exon (Supplementary Figure 1) [4]. The deletion frameshift *IFNL4*-ΔG allele creates an open reading frame that allows production of a novel IFN, IFN-λ4, which cannot be produced in individuals homozygous for the *IFNL4*-TT allele (*IFNL4*-TT/TT) [4]. *IFNL4*-ΔG is in linkage disequilibrium (LD) with the unfavorable rs12979860-T allele, although the extent of LD differs by racial background [4]. In patients of African or European ancestry, LD between these markers is moderate and *IFNL4*-ΔG genotype predicts slower viral decline and lower odds of SVR to IFN-α/RBV treatment better than rs12979860 [4, 5]. In individuals of Asian ancestry, the variants are in near complete LD and are expected to provide similar predictive information.

Despite the strong predictive value of both *IFNL4* variants for response to IFN-α/RBV, their relevance for response prediction to IFN-α–free DAA therapy is unclear [3]. Recently, we treated treatment-naive, HCV genotype-1 patients with the DAA sofosbuvir, an NS5B inhibitor, and RBV for 24 weeks in a phase 2a clinical trial [6]. Patients had a high prevalence of

predictors of poor treatment response to IFN-α/RBV therapy, including black race (83%), obesity (52%), HCV genotype-1a (70%), high HCV RNA (62%), unfavorable rs12979860 TT or CT genotype (81%), and advanced liver disease (22%) [6].

Overall, 38 of 55 patients achieved an SVR at 24 weeks post treatment (SVR_{24}), while 17 patients relapsed after treatment [6]. A mixed VK–pharmacokinetic (VK–PK) model in a subset of patients showed faster loss of free virus and higher drug effectiveness in SVR_{24} patients as compared with relapsers [6]. However, in a fully fitted VK–PK model, rs12979860 genotype (CC vs CT and TT rs12979860 genotypes) did not predict viral decline.

Because *IFNL4*-ΔG genotype appears to predict response to IFN-α–based treatment better than rs12979860, particularly in African Americans who comprise 83% of the patients in our trial, we analyzed the association of *IFNL4*-ΔG with virological response parameters. We observed that patients who carry at least 1 *IFNL4*-ΔG allele have significantly slower early viral clearance compared with patients who do not carry this allele (*IFNL4*-TT/TT homozygotes).

EXPERIMENTAL PROCEDURES

Clinical Trial Design

As previously described, treatment-naive, chronic HCV patients infected with HCV genotype 1 were treated for 24 weeks with sofosbuvir (Gilead Sciences, Foster City, CA) plus low-dose or weight-based RBV (clinicaltrials.gov identifier NCT01441180) [6]. The National Institute of Allergy and Infectious Diseases Institutional Review Board–approved written or oral informed consent was obtained from all participants. Of 60 patients who enrolled, 54 patients completed 24 weeks of therapy, 1 patient completed 12 weeks, and 5 patients dropped out of the study before week 8 [6]. Twenty-five patients participated in a VK–PK substudy, as previously described, with 1 dropout at week 3 [6]. Of these patients, 20 were African American, 4 were white, and 1 was Hispanic by self-report. Ten patients received low-dose RBV while 15 received weight-based RBV.

Viral Kinetics

Levels of sofosbuvir and its active metabolite GS-331007 were measured using high-performance liquid chromatography–mass spectrometry (QPS, LLC, Newark, Deleware) at 0, 1, 2, 4, 8, 12, 24, and 36 hours after administration. Plasma HCV RNA levels were measured at hours 0, 1, 2, 4, 8, 12, and 24; days 3, 5, 7, 10, and 14; and weeks 3 and 4 by quantitative reverse-transcriptase polymerase chain reaction (Abbott Laboratories, Abbott Park, IL) with a lower limit of quantification of 12 IU/mL and a lower limit of detection of 3 IU/mL.

Genetic Analysis

Genotyping of *IFNL4* variants rs12979860 and rs368234815 was performed on genomic DNA with custom TaqMan assays as previously described [4]. Haploview 4.2 was used to estimate haplotype frequencies and LD between the *IFNL4* markers (D′ and r^2) [7].

VK–PK Model

VK, PK, and pharmacodynamic data for the 25 patients were fitted using a VK–PK model as previously described [8] and as used by Osinusi et al [6]. In brief, a Bateman function was used to fit the levels of GS-331007 through hour 36. Afterward, an ordinary differential equation system was used to describe viral kinetics and treatment effects of RBV and sofosbuvir. In contrast to the model used in [8], we used a slightly different association between drug levels and antiviral efficacy ε. As levels of GS-331007 are highly variable, the treatment efficacy factor ε of blocking viral production of sofosbuvir was assumed to depend, through the Hill function, only indirectly on levels of GS-331007 through an intermediate compartment Z, calculated as $dZ/dt\ (t) = a(C(t) - Z(t))$. In this equation, C is the concentration of GS-331007 and a is the rate linking the drug concentration compartment, as it is quantified, and the effectively active drug compartment. With this approach, treatment efficacy depends on PK of GS-331007, but the variation is slightly damped and delayed. Log HCV RNA levels over the first 30 days of treatment were fitted using a maximum likelihood approach that accounts for data below the quantitation limits [9]. We did not use a delay parameter t_0, as this is unnecessary with this model, which already allows a delay in drug effectiveness by the PK of GS-331007 as well as by the intermediate compartment Z.

Statistical Analysis

To assess potential differences in viral kinetics by *IFNL4*-ΔG or rs12979860 genotypes, fitted model parameters were compared with the Jonckheere and Terpstra trend test, which accounts for the ordinal scale of the genotypes and provides a general P value without the need for a multiple test correction.

RESULTS

Baseline characteristics and treatment outcomes for the 25 patients analyzed in the VK–PK substudy were representative of all patients enrolled in the study (Supplementary Table 1). Due to the overall high LD between *IFNL4* variants rs12979860 and rs368234815 in the full patient set ($n = 59$, $D' = 1.0$, $r^2 = 0.87$) and the VK–PK subset ($n = 25$, $D' = 1.0$, $r^2 = 0.85$), in most patients the CC, CT, and TT genotypes of rs12979860 corresponded to the TT/TT, TT/ΔG, and ΔG/ΔG genotypes of rs368234815 (Supplementary Table 2). Four patients were discordant for these 2 markers; 2 had a favorable CC rs12979860 genotype but an unfavorable heterozygous TT/ΔG rs368234815 genotype (1 dropout, 1 relapser), while 2 patients had an

unfavorable heterozygous CT rs12979860 genotype and homozygous ΔG/ΔG rs368234815 genotype (1 SVR_{24}, 1 relapser). All 4 patients with discordant genotypes were African Americans, which is consistent with lower LD between these markers in individuals of African ancestry [4].

In a fully fitted VK–PK model, the loss rate of free virus (*c*) was slower for carriers of *IFNL4*-ΔG (*IFNL4*-ΔG/ΔG, $c = 4.9$; *IFNL4*-TT/ΔG, $c = 4.7$) than for patients who do not carry this variant (*IFNL4*-TT/TT, $c = 5.8$; $P = .039$; Figure 1*A*, Table 1). Drug efficacy (ε) during early treatment also varied by genotype (*IFNL4*-ΔG/ΔG, $\varepsilon_{mean} = 0.93$; *IFNL4*-TT/ΔG, $\varepsilon_{mean} = 0.97$; *IFNL4*-TT/TT, $\varepsilon_{mean} = 0.98$; $P = .048$; Table 1), but the loss rate of infected cells (delta) did not (*IFNL4*-ΔG/ΔG, delta = 0.16; *IFNL4*-TT/ΔG, delta = 0.20; *IFNL4*-TT/TT, delta = 0.19; $P = .31$; Table 1). Similar results were obtained when the analysis was restricted to the 20 African-American patients (data not shown). Notably, classification of *IFNL4* genotype using the rs12979860 SNP did not show significance for *c* ($P = .13$), although ε retained significance ($P = .027$; Figure 1*B*, Table 1).

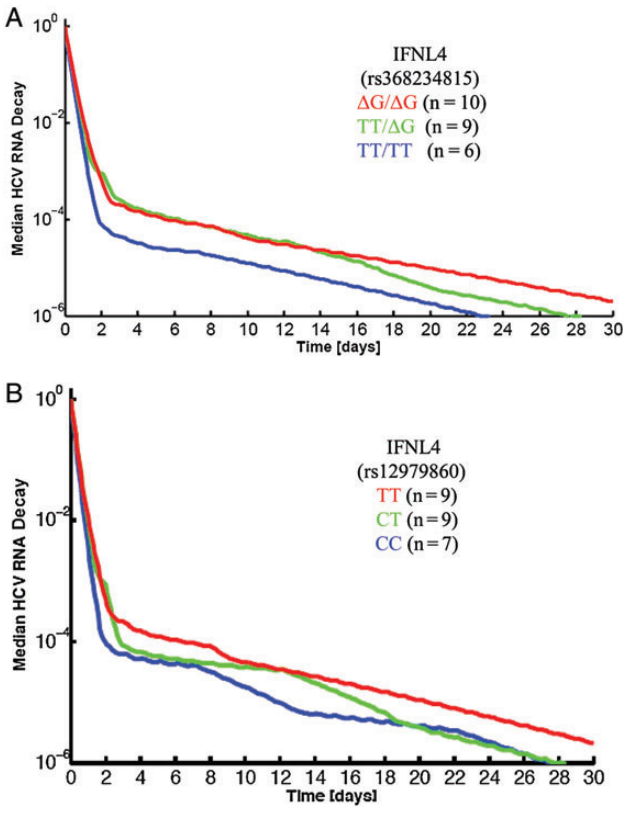

Figure 1. Viral kinetic (VK) decline, determined by a fully fitted VK-PK (pharmacokinetic) model of hepatitis C virus (HCV) treatment with sofosbuvir/ribavirin, is affected by *IFNL4* genotype as determined by (*A*) rs368234815 and (*B*) rs12979860. As shown in Figure 1*A*, slower HCV viral decline is associated with the *IFNL4*-ΔG rs368234815 allele as compared with other alleles.

Seven of 8 patients (87.5%; 95% confidence interval [CI], 47.4%–99.7%) with the *IFNL4*-TT/TT genotype achieved SVR_{24} as compared with 31 of 47 with TT/ΔG or ΔG/ΔG genotypes (66.7%; 95% CI, 51.7%–77.8%), but this difference was not statistically significant in this small study ($P = .4$, Fisher exact test).

DISCUSSION

Genotypes of 2 genetic markers within a novel *IFNL4* gene—an intronic SNP rs12979860, previously referred to as an *IFNL3* (*IL28B*) variant, and an exonic IFN-λ4 protein-creating variant, rs368234815—are predictive of VK decline and treatment outcome for chronic HCV patients treated with IFN-α/RBV therapy [4, 10]. Here we show a significant association of *IFNL4*-ΔG genotype with slower early viral decline for HCV genotype-1–infected patients treated with the IFN-α–free DAA regimen of sofosbuvir/RBV. A fully fitted VK–PK model showed that patients carrying at least 1 *IFNL4*-ΔG allele had significantly slower loss rate of free virus (*c*) and drug efficacy (ε) compared with patients homozygous for the *IFNL4*-TT allele.

Although this trial had insufficient statistical power for a meaningful analysis of the association between *IFNL4*-ΔG genotype and SVR, a previous analysis revealed slower early VK decline in patients who later relapsed [6]. Together, these data suggest that *IFNL4*-ΔG genotype, which affords the ability to make IFN-λ4, impacts viral decline and could affect treatment outcome for this DAA-based regimen.

Consistent with our findings, rs12979860 genotype was associated with favorable treatment outcome for another DAA regimen in HCV genotype-1 infection (faldaprevir, deleobuvir, and RBV) [11], suggesting the effect may not be regimen specific. Phase 3 trials of sofosbuvir/RBV with or without IFN-α for HCV genotypes 1, 2, and 3 were recently conducted, and while an association of rs12979860-CC genotype was found in the NEUTRINO study in genotype-1–infected patients treated with sofosbuvir and IFN-α/RBV, no association with treatment outcome was observed in the IFN-α–free arms in genotype-2 and -3–infected patients [12, 13]. The predictive value of *IFNL4* genetic variants for IFN-α–free DAA therapies requires further assessment in larger trials.

VK decline in association with rs12979860 was previously assessed during DAA therapy using a biphasic model rather than the fully fitted model used here. Chu et al found a mixed effect on phase 1 decline with slower velocity but longer duration in patients with the rs12979860-CC genotype who were treated with mericitabine and danoprevir for 14 days [14]. In contrast, our findings show slower early viral decline with reduced slope and magnitude for patients carrying at least 1 *IFNL4*-ΔG allele in comparison with *IFNL4*-TT/TT homozygotes. Differences in our findings may be explained by the use

Table 1. Association of *IFNL4* Variants With Viral Kinetic Decline and Pharmacokinetics

	rs368234815			
Parameter	TT/TT (n = 6)	TT/ΔG (n = 9)	ΔG/ΔG (n = 10)	*P* Value
c	5.8 (5.2–6.1)	4.7 (2.2–7.1)	4.9 (1.8–5.8)	**.039**
Delta	0.19 (0.02–0.40)	0.20 (0.02–0.38)	0.16 (0.12–0.43)	.31
Epsilon mean	0.98 (0.88–0.99)	0.97 (0.68–0.99)	0.93 (0.69–0.98)	**.048**
Epsilon max	0.98 (0.90–0.99)	0.98 (0.73–0.99)	0.94 (0.75–0.98)	**.048**
GS-7977 AUC	84 (38–151)	97 (41–169)	73 (50–146)	.48
GS-331007 AUC	517 (252–1070)	502 (276–1035)	662 (513–793)	.087
	rs12979860			
Parameter	CC (n = 7)	CT (n = 9)	TT (n = 9)	*P* Value
c	5.7 (3.5–6.1)	4.7 (2.2–7.1)	5.1 (1.8–5.8)	.13
Delta	0.23 (0.02–0.40)	0.16 (0.02–0.38)	0.17 (0.12–0.43)	.31
Epsilon mean	0.98 (0.88–0.99)	0.98 (0.68–0.99)	0.92 (0.69–0.97)	**.027**
Epsilon max	0.98 (0.90–0.99)	0.98 (0.73–0.99)	0.94 (0.75–98)	**.027**
GS-7977 AUC	77 (38–151)	97 (41–169)	74 (51–146)	.30
GS-331007 AUC	476 (252–1070)	574 (344–1035)	689 (513–793)	**.034**

Loss of free virus (*c*) per day, drug efficacy (epsilon mean and max), and loss rate of infected cells (delta) per day are shown for *IFNL4* variants rs368234815 and rs12979860. GS-7977 (pro-drug) and GS-331007 (active metabolite) area-under-the-curve (AUC) measures are shown. Significant *P* values (<.05) are bolded. Values in the table represent medians with range in parentheses.

of a mechanistic and fully fitted VK model rather than a descriptive biphasic model or they may reflect differences in the relative potencies of the DAA regimens.

It is unclear how IFN-λ4 impairs response to IFN-α–containing and IFN-α–free treatment regimens. Similar to other members of the IFN-λ family, IFN-λ4 signals through the IFN-λ receptor complex, induces expression of IFN-stimulated genes (ISGs) via JAK-STAT signaling, and has antiviral properties, yet IFN-λ4 differs in that it is poorly secreted [4, 15]. We hypothesize that in individuals who carry *IFNL4*-ΔG, induced IFN-λ4 expression may contribute to persistent hepatic ISG activation, which could have a negative cross-regulatory effect on the immunological response to HCV infection.

Strengths of this study include the detailed VK–PK data collected in 25 patients, as well as data on the recently discovered *IFNL4*-ΔG variant. The main limitation of the study is the small number of participants, which precludes the ability to assess the effect of *IFNL4* genotype on achieving SVR.

In summary, we provide evidence that the *IFNL4*-ΔG allele is associated with slower early VK decline for the IFN-α–free regimen of sofosbuvir/RBV in HCV genotype-1–infected patients. Knowledge of *IFNL4* genotype could potentially be useful for guiding duration or intensity of successful DAA therapy. The biological mechanism by which IFN-λ4 affects the response to IFN-α–containing and IFN-α–free treatment of HCV is under investigation. The impact of *IFNL4* genotype and IFN-λ4 protein on HCV clearance merits testing in subsequent DAA studies with incomplete treatment responses.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank Gilead Sciences for provision of sofosbuvir.

Financial support. This work was supported in part, with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN261200800001E, the National Institutes of Health Clinical Center, and the Division of Cancer Epidemiology and Genetics of the National Cancer Institute. This research was supported, in part, by the intramural program of the National Institute of Allergy and Infectious Diseases. The study was also supported, in part, by the German Research Foundation (DFG) by the clinical research unit KFO 129.

Potential conflicts of interest. L. P. -O. and T. R. O'B. are inventors on patent applications filed by the National Cancer Institute for the *IFNL4*-ΔG (rs368234815) genotype-based test and for the IFN-λ4 protein. E. H. served as a research consultant of Roche Pharma and Novartis.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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