**IL28B Genotype Is Associated With Differential Expression of Intrahepatic Interferon-Stimulated Genes in Patients With Chronic Hepatitis C**

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Genetic variation in the IL28B (interleukin 28B; interferon lambda 3) region has been associated with sustained virological response (SVR) rates in patients with chronic hepatitis C (CHC) who were treated with peginterferon-α and ribavirin. We hypothesized that IL28B polymorphism is associated with intrahepatic expression of interferon-stimulated genes (ISGs), known to influence treatment outcome. IL28B genotyping (rs12979860) and whole-genome RNA expression were performed using liver biopsies from 61 North American patients with CHC. After correction for multiple testing (false discovery rate < 0.10), 164 transcripts were found to be differentially expressed by IL28B-type. The interferon signaling pathway was the most enriched canonical pathway differentially expressed by IL28B-type (P < 10⁻⁵), with most genes showing higher expression in livers of individuals carrying the poor-response IL28B-type. In 25 patients for which treatment response data were available, IL28B-type was associated with SVR (P = 0.0054). ISG expression was also associated with SVR; however, this was not independent of IL28B-type. Analysis of miR-122 expression in liver biopsies showed reduced miR-122 levels associated with poorer treatment outcome, independently of IL28B-type. No association was observed between IL28B-type and levels of liver IL28B or IL28A messenger RNA expression. IL28B protein sequence variants associated with rs12979860 were therefore investigated in vitro: no differences in ISG induction or inhibition of HCV replication were observed in Huh7.5 cells. Conclusion: The good response IL28B variant was strongly associated with lower level ISG expression. The results suggest that IL28B genotype may explain the relationship between hepatic ISG expression and HCV treatment outcome, and this is independent of miR-122 expression. IL28B-type was not associated with intrahepatic IL28B messenger RNA expression in vivo. Further investigation of the precise molecular mechanism(s) by which IL28B genetic variation influences HCV outcomes is warranted. (HEPATOLOGY 2010;52:1888-1896)

Three initial independent genome-wide association studies (GWAS) have recently identified variation in the region of the IL28B gene on chromosome 19 to be a key predictor of peginterferon-α (pegIFN) and ribavirin (RBV) treatment response in patients chronically infected with genotype 1 hepatitis C virus.¹⁻³ This region was subsequently also identified to be important for spontaneous clearance of HCV infection,⁴⁻⁵ suggesting a role in the regulation of endogenous as well as exogenous type 1 IFN responses. However, the causal variant and the biological mechanism responsible for this association have yet to be unraveled.

**Abbreviations:** ANOVA, analysis of variance; CHC, chronic hepatitis C; GWAS, genome-wide association study; HCV, hepatitis C virus; IFN, interferon; IL28A, interleukin 28A; IL28B, interleukin 28B; ISG, interferon-stimulated gene; PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism; SVR, sustained virological response.

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In addition, in a GWAS analysis of pretreatment HCV viral load, the paradoxical observation was made that the good response variant was associated with a higher level of circulating HCV RNA. Although the differences in median viral load between the respective *IL28B* genotypes were small and perhaps of limited clinical significance, the finding was surprising, given that most therapeutic studies have noted an association between high viral load and reduced response to pegIFN and RBV treatment. A reasonable hypothesis might involve differential levels of intrahepatic interferon-stimulated gene (ISG) expression according to *IL28B* genotype, where the good response variant was associated with lower levels of intrahepatic ISG expression, and therefore slightly higher levels of circulating serum HCV RNA.

There has been recent interest in the relationship between intrahepatic ISG expression in patients chronically infected with HCV, and response to IFN-based treatment regimens. A growing body of evidence, in both humans and the chimpanzee model, supports a paradigm in which IFN nonresponse is associated with pre-activated, perhaps maximally or perhaps inappropriately stimulated intrahepatic ISG expression. In contrast, quiescent liver ISGs pretreatment have been associated with sensitivity to exogenous IFN therapy and viral eradication. The mechanistic basis for this association is unknown, and it remains unclear whether it is driven by the host, the virus, or both.

We hypothesized that these patterns of intrahepatic ISG expression reflect differences in host innate antiviral immunity, and that a key determinant is the recently identified *IL28B* polymorphism. The current analysis summarizes results of global liver gene expression profiling according to *IL28B* genotype, in a well-characterized cohort of North American chronic hepatitis C patients, and *in vitro* assays of cytokine potency according to *IL28B* genotype.

**Patients and Methods.** Sixty-one patients were recruited for this study; all attended the Liver Clinic at Beth Israel Deaconess Medical Center (Harvard University, Boston, MA) for the management of HCV mono-infection, and had consented to storage of liver biopsy specimens and clinical data for research purposes. Liver biopsy in all patients was performed at this institution, and liver specimens were immediately placed in RNAlater (Qiagen, Valencia, CA) before storage at -80°C. Forty-one patients were treatment-naïve at the time of liver biopsy, and 20 were nonresponders (NR) to prior IFN-based treatment regimens (standard/pegIFN ± RBV). Detailed description of initial viral response was not available for all patients, and because sample size was small, we grouped nonresponders and relapers as treatment failures and compared them to patients who attained SVR (responders). All patients had stopped antiviral therapy at least 6 months prior to biopsy. Written informed consent was obtained from all patients. The protocol was approved by the Institutional Review Board at Beth Israel Deaconess Medical Center.

At the time of liver biopsy, all patients had blood taken for serum liver function tests, a quantitative measure of serum/plasma HCV RNA performed using the Roche polymerase chain reaction (PCR) assay, with a lower limit of detection of 50 IU/mL. HCV genotyping was performed using the line probe assay in all patients. All biopsy samples were evaluated by a single hepatopathologist and scored for stage of fibrosis and grade of inflammation using the METAVIR scoring system.

**DNA/RNA Extraction.** Total RNA and genomic DNA were extracted from liver biopsies using the Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA) with a modification of the manufacturer's protocol for DNA extraction after elution of RNA (www.natureprotocols.com/2009/08/17/a_useful_procedure_to_isolateRNA.php).

**IL28B Genotyping.** The region on chromosome 19 associated with HCV treatment response contains multiple single-nucleotide polymorphisms (SNPs) in linkage disequilibrium around the *IL28B* gene. We selected the most strongly associated SNP, rs12979860, which is a bi-allelic polymorphism (CC, CT, TT) that has been associated with treatment response in both Caucasians and African Americans. Genotyping was performed using the ABI TaqMan allelic discrimination kit and the ABI7900HT sequence Detection System (Applied Biosystems, Carlsbad, CA) in 5 μL reaction volume using standard TaqMan Universal PCR conditions.
and probe sequences were: Forward Primer 5'-GCCTGTCTGTACTGAACCA-3', Reverse Primer 5'-GGCGGGAATGGCATTCAAC-3', Probe (C allele) 5'-VIC-TGGTTCGCGCTTC-3', Probe (T allele) 5'-FAM-CTGGTTCGCGCTTC-3'. Genotyping was conducted in a blinded fashion relative to HCV treatment status and other patient or treatment response characteristics.

**Expression Analysis.** Approximately 200 ng total RNA was amplified and transcribed to cRNA, and hybridized to Illumina HT-12 Expression BeadChip per the manufacturer’s protocol (Illumina Inc., San Diego, CA), targeting approximately 25,000 annotated genes with more than 48,000 probes.

**Quantitative PCR for Targeted Gene Expression Measurements.** A custom TaqMan MGB assay for gene expression was designed to achieve 1000-fold specificity for IL28B versus the close homolog IL28A (Supporting Fig. 1). Primer and probe sequences are provided in the Supplemental Materials. Applied Biosystems TaqMan Assays-on-Demand were used to detect expression of selected ISGs: IFI6, IFI27, and ISG15. miR-122 and RNA U6 determination was made using a specific TaqMan MicroRNA Assay (Applied Biosystems, Foster City, CA, USA). Approximately 1 μg of total RNA from liver biopsies was reverse transcribed using the High-Capacity cDNA Synthesis Kit (Applied Biosystems, Foster City, CA, USA), using either an miRNA-specific primer (for miR-122 and U6) or random hexamer primers. Approximately 100 ng cDNA was used per reaction in a total volume of 10 μL, and duplicate reactions were performed for each sample. Data were transferred using the ΔΔCT method normalizing to GAPDH or beta-actin as reference genes (for miR-122, U6 RNA was used as a reference), and further normalized to the lowest-expressing sample for comparison.

**In Vitro Characterization of Coding IL28B Variants.** Recombinant proteins were produced by cloning expression-optimized (Blue Heron Biotechnology) IL28B cDNA sequences corresponding to the two alternative alleles at the rs8103142 IL28B coding variant into pGEX-6P-2. GST fusion proteins were expressed in BL-21 cells and purified using glutathione-agarose according to the manufacturer (GE Healthcare). Protein integrity and concentration were determined by SDS-PAGE.

For purification from human cells, the IL28B cDNA sequences corresponding to the two alternative alleles at the rs8103142 variant were cloned into pcDNA5/FRT/TO and stably integrated into Flp-in-Trex 293 cells (Invitrogen, Carlsbad, CA). After 24 hours of culture of confluent stable cells in serum-free media containing 1 μg/mL doxycycline, the supernatant was collected and concentrated using centricron devices (Millipore, Billerica, MA). Total protein was measured by the Bradford method, and serial dilutions of concentrate were used to treat Huh7.5 cells hosting an HCV replicon as described previously. HCV replicon RNA measurement was performed using a custom TaqMan real-time PCR method, normalizing to GAPDH messenger RNA (mRNA) expression. Primer and probe sequences for HCV RNA determination were: Forward Primer 5'-TCAATAGGGTGCTTT CATGCCCTCA-3', Reverse Primer 5'-TGAGGTGA GTTTGACGTGTTTCCT-3', Probe 5'-VIC-TGGCA AGTACCTCTTTCAACTGGGCA-3'. Measurement of MX1 expression was performed in parallel using a specific TaqMan Gene Expression Assay (Applied Biosystems, Foster City, CA). Kinetics of HCV inhibition and MX1 stimulation were estimated by least-squares regression using GraphPad Prism version 5 (GraphPad Software, Inc., La Jolla, CA).

**Statistical Analysis.** Microarray expression data were analyzed using the Partek Genomics Suite version 6.4 (Partek, Inc., St. Louis, MO). Expression data were log2-transformed and normalized by quantile normalization prior to statistical analysis. Outlier samples identified by principal components analysis were removed from the study. A linear mixed model analysis was performed using analysis of covariance. IL28B genotype, sex, age, and ethnicity were included in the model. A false discovery rate threshold of 0.1 was used to correct for multiple testing, corresponding to a P value < 4.3 × 10^{-4}. Baseline HCV viral load was added to the linear model to determine viral load-independent relationships between IL28B genotype and gene expression. Enrichment of differentially expressed genes in particular biological pathways was determined using Ingenuity Pathway Analysis v8.0 (Ingenuity Systems, Inc., Redwood City, CA). A composite ISG metric was calculated as the average expression level of IFI6, IFI27, and ISG15 after linear (anti-log2) transformation and tested for association with SVR, IL28B genotype, and miR-122 expression by analysis of variance (ANOVA). Differences in potency between IFN-α3 variants were assessed by comparison of the 95% confidence intervals of the IC_{50} or EC_{50} parameter estimates.

**Results**

**Patient Characteristics.** The characteristics of the 61 patients included in the study are described in Table 1. Patients represented multiple ethnicities...
including African American, Caucasian and Hispanic, with the majority infected with genotype 1 HCV (Table 1). Most patients had mild-to-moderate hepatic necroinflammatory activity and fibrosis. The distribution of IL28B genotype is listed in Table 1. The C allele frequency was 0.55 and these genotypes were in Hardy-Weinberg equilibrium ($P = 0.76$).

**Gene Expression Profile Versus IL28B Genotype.** Based on previous reports suggesting modest differences between CT and TT genotypes at rs12979860 in relation to SVR,\(^1\-^3\) genotypes were collapsed into a recessive model and comparisons were performed as CC versus CT or TT (non-CC) genotypes. After applying a false discovery rate threshold of $<0.1$ to minimize false positive associations, a total of 164 genes were found to be differentially expressed between genotype groups. A full list of differentially expressed genes and the corresponding fold-change in expression and $P$ value is provided in Supporting Table 1.

Further filtering to those genes with false discovery rate (FDR) $< 0.1$ and absolute expression differences of at least $\pm 1.5$-fold between CC versus non-CC individuals gave a subset of 32 genes highly differentially expressed by genotype. Hierarchical clustering of this subset showed strong discrimination by IL28B genotype (Fig. 1), with a large number of ISGs having significantly lower expression in CC versus non-CC genotypes, including biologically relevant ISGs such as MX1 (2.6-fold), OAS1 (1.9-fold), OAS2 (2.2-fold), OAS3 (2.1-fold), IFIT1 (2.3-fold), IFIT2 (1.7-fold), IFIT3 (1.8-fold), and ISG15 (3.9-fold). Notably, three genes in this subset showed the reverse effect, with higher expression in CC versus non-CC individuals: FCN1 (1.8-fold), CXCL9 (3.3-fold), and CCL8 (1.6-fold).

Genes showing significant association with IL28B genotype were then analyzed for overrepresentation in known biological pathways using Ingenuity software. A number of immunological pathways were found to be significantly enriched for IL28B-associated genes, including interferon signaling as well as viral recognition pathways and a variety of adaptive and innate immune response pathways (Fig. 2). Further analysis of enrichment for biological functions showed a similar enrichment for inflammatory and antimicrobial response genes, among other functions such as cell-to-cell signaling (Supporting Fig. 2).

**Tests for Independence of IL28B-Associated and Viral Load-Associated ISG Expression.** It was hypothesized that high baseline viral load may induce ISG expression in a manner unrelated to IL28B genotype. Thus, further analysis was performed to determine whether particular genes may be associated with IL28B genotype independently of viral load. Inclusion of baseline viral load in the ANOVA model revealed essentially the same set of genes associated with IL28B genotype, i.e., that the differences in gene expression by IL28B genotype were largely independent of HCV viral load at baseline. Hierarchical clustering of gene expression showed a similar pattern of low ISG expression in CC individuals, with only FCN1 and CXCL9 showing higher expression in the protective CC genotype group (Supporting Fig. 3).

**Tests for Independence of IL28B Genotype and ISG Expression in Prediction of Treatment Response.** Data on pegIFN and RBV treatment response (sustained virologic response, or SVR) was available for a small subset of patients ($n = 25$, including five responders and 20 nonresponders). 17 were prior nonresponders to pegIFN and RBV therapy (all treatment had been stopped at least 6 months prior to liver biopsy); 8 were treated after the liver biopsy, and of these patients, 5 were responders and 3 were nonresponders. This allowed for an exploratory analysis of the relationship between ISG expression and SVR conditioned on IL28B genotype. Despite the small sample size, a significant association between CC genotype and SVR was observed in this subset ($P = 0.0054$, CC versus non-CC). In the same subset of samples, tests for association between SVR and 3 ISGs previously shown to be relevant to treatment outcome (ISG15, IFI27 and IFI16) were shown to be significant. These genes were chosen on the basis of previous data linking them to SVR,\(^7\) and all 3 showed at least a 1.5-fold difference in expression between responders and nonresponders.

### Table 1. Clinical Characteristics and Genotype Distributions in the Study Cohort ($n = 61$)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (range/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, in years</td>
<td>51 (43-56)</td>
</tr>
<tr>
<td>Male sex</td>
<td>41 (67.21%)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>38 (62.30%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>5 (8.20%)</td>
</tr>
<tr>
<td>Asian</td>
<td>5 (8.20%)</td>
</tr>
<tr>
<td>African American</td>
<td>12 (19.67%)</td>
</tr>
<tr>
<td>HCV RNA (log10 IU/mL)</td>
<td>6.08 (5.61-6.62)</td>
</tr>
<tr>
<td>ALT (IU/mL)</td>
<td>63 (46-87)</td>
</tr>
<tr>
<td>METAVIR activity A2-3 (n,%)</td>
<td>32 (53.33%)</td>
</tr>
<tr>
<td>METAVIR fibrosis F3-4 (n,%)</td>
<td>2 (3.33%)</td>
</tr>
<tr>
<td>IL28B genotype</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>19 (31.15%)</td>
</tr>
<tr>
<td>CT</td>
<td>29 (47.54%)</td>
</tr>
<tr>
<td>TT</td>
<td>13 (21.31%)</td>
</tr>
<tr>
<td>C allele frequency</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Data represent median (25th-75th percentile) unless otherwise indicated.
Fig. 1. Hierarchical clustering of samples according to genes showing the greatest differences in expression between IL28B genotypes. For clarity, only probes with FDR-corrected $P$ value $<0.1$ and a fold-change of $>1.5$ or of $<-1.5$ were included in the dendrogram. IL28B-CC samples have higher expression than CT and TT samples when probe is red and lower expression when probe is blue.

Fig. 2. Pathway analysis showed that genes showing differential expression by IL28B-type clustered in the canonical interferon signaling pathway (FDR $P$ value threshold $= 0.1$). Several additional immune-related pathways reach significance in this analysis.
In the current data set, and were confirmed by real-time quantitative PCR. A composite ISG expression variable based on the average value of the real-time PCR-based expression measures for these three ISGs similarly showed a significant association with SVR. Because of limitations of sample size, we lacked sufficient power to discriminate whether ISG expression or IL28B genotype had stronger association with SVR; however, an exploratory analysis including both IL28B genotype and mean ISG expression in the regression model suggested that IL28B genotype may be the predominant factor (Supporting Table 2).

**miR-122 Expression and IL28B Genotype.** Recent evidence indicates a necessary role of miR-122 expression in HCV replication. Therefore, we evaluated the relationship between IL28B type and intrahepatic expression levels of miR-122 using a targeted real-time PCR assay (miRNA expression levels were not tested on the microarray). miR-122 expression showed no correlation with rs12979860 genotype (Supporting Fig. 4). In relation to SVR, miR-122 expression showed significantly lower expression levels in liver biopsies from nonresponders compared with responders (Fig. 3), as described. Furthermore, although there was a high degree of correlation among the targeted ISGs IFI6, IFI27 and ISG15, these ISGs were not correlated with miR-122 expression (Supporting Fig. 5), suggesting that the association between IL28B-type, ISG expression levels and SVR is independent of miR-122.

**Intrahepatic Expression of IL28B.** Expression of IL28A and IL28B mRNA was detectable in HCV infected liver tissue; however, expression of these transcripts showed no significant relationship with IL28B genotype. Because of the lack of specificity in the probe sequences for IL28A versus IL28B on the microarray, and the superior quantitative power of targeted real-time PCR assays, a specific quantitative PCR assay was designed for determination of IL28B mRNA expression (Supporting Fig. 1). Consistent with the microarray results, IL28B mRNA expression as determined using the targeted assay showed no relationship with IL28B genotype (Fig. 4). Additionally, no relationship was observed between IL28B genotype and liver IL28A mRNA expression (Supporting Fig. 6).

**Comparison of Variant IL28B Potencies In Vitro.** We have previously identified through fine mapping strategies that the rs12979860 polymorphism is on a common haplotype block with a nonsynonymous coding variant in exon 2 of IL28B, rs8103142, the only coding polymorphism in IL28B. As described previously, the set of polymorphisms identified in the IL28B gene region are highly correlated (r² > 0.85 for all pairwise comparisons), and therefore it is not possible through genetic association alone to determine which if any of these variants is causal for the association, given current sample sizes. Because no significant differences were detected in IL28B mRNA expression levels in CC versus non-CC individuals (Fig. 4), we reasoned that the single conservative amino acid change (Lys70Arg, rs8103142) correlating with IL28B genotype may be responsible for differential antiviral potency and induction of ISGs. To test this hypothesis, the recombinant IFN-λ3 variants were expressed in E. Coli as GST-fusion proteins and purified for functional assays. Treatment of Huh7.5 hepatoma cells harboring the HCV-Con1 subgenomic
replicon with either IFN-κ3 variant caused similar dose-dependent declines in viral RNA abundances, with maximum inhibitory concentrations of ~10 ng/mL (Fig. 5). Corresponding analysis of MX1 mRNA levels demonstrated potent MX1 induction after treatment with either variant, suggesting that the Lys to Arg amino acid difference does not affect IFN-κ3 potency. IFN-κ3 is not known to undergo post-translational modifications that would be absent in recombinant proteins produced in E. Coli. Nevertheless, we wished to exclude the possibility that a hypothetical difference in potency due to Lys70Arg would be obscured due to expression in bacteria. We therefore established stable HEK-293 cell lines that inducibly express each IL28B variant and secrete the IFN-κ3 proteins into cell culture media. In order to test functionality of these factors, media from cells expressing IL28B were harvested and concentrated and, as a negative control, media from parental cells lacking a stable IL28B transgene was similarly concentrated. Western blot analysis revealed that IFN-κ3 was only detectable in media from overexpressing cells (data not shown). HCV replicon-harboring cells were treated with varying amounts of IFN-κ3 or control media and measurements of HCV and MX1 RNA levels were again performed. As with results obtained with E. Coli-produced proteins, each IFN-κ3 variant generated in human cell culture significantly repressed HCV RNA levels and induced MX1 expression with indistinguishable potencies (data not shown). Taken together, these data suggest that that other noncoding variant(s) dictate phenotypic response to anti-HCV therapy.

Discussion

We have investigated the relationship between IL28B genotype and patterns of intrahepatic gene expression in an ethnically diverse U.S.-based cohort of patients chronically infected with HCV. Previous reports have shown that high baseline ISG expression in liver tissue is associated with a poor response to treatment with IFN-based therapy. Here, we show that IL28B genotype is strongly associated with intrahepatic ISG expression, with the poor-response (non-CC) IL28B genotypes exhibiting higher ISG levels compared to patients with the favorable CC IL28B genotype. In a subgroup, viral clearance was associated with both IL28B genotype and lower level ISG expression. We also show that liver expression levels of IL28B mRNA are not associated with IL28B genotype, suggesting that the polymorphism might have an effect in biological potency of IL28B signaling. However, experimental analysis of the nonsynonymous coding variant rs8103142 did not demonstrate any differences in anti-HCV or ISG induction potency between protein sequence variants.

The analysis of the intrahepatic transcriptome in patients with the poor response CT/TT IL28B-types compared to CC patients identified differential expression of >150 genes. The list was dominated by canonical ISGs, including MX1, OAS1/2/3, IFIT1/2/3, IFI6/27/44 and ISG15; all were up-regulated >1.5-fold in CT/TT patients compared to CC patients. These genes have previously been shown to be among the main effector pathways of the IFN-mediated antiviral response. Pathway analysis confirmed that genes showing differential expression by IL28B-type clustered in the canonical interferon signaling pathway. That baseline induction of high level ISG expression is associated with poor therapeutic response to exogenous IFN therapy, perhaps by exhausting the IFN response
pathway, has been suggested previously. The data therefore confirms, in a North American population, the recent results from a study in a Japanese patient population reporting an association between intrahepatic levels of ISG expression and pegIFN/RBV response as well as IL28B genotype (rs8099917). Among the transcripts whose expression was highly differentially by IL28B genotype, only three genes (CCL8, CXCL9, and FCN1) were shown to be significantly up-regulated in the protective CC genotype group. The chemotactic cytokine (CC) pathway, and CXCL9 in particular, has previously been implicated in the control of HCV infection. The ficolin-1 (FCN1) gene, though not previously associated with anti-HCV activity, is suspected to play a role in innate immunity and also represents a plausible candidate for control of HCV.

The explanation for the discordant expression of these 3 genes relative to the general pattern of low level ISG expression remains unclear. A key question is whether the pattern of intrahepatic ISG expression is driven directly by IFN-α, is a secondary effect of IFN-α modulating a type 1 IFN effect, and this will require further investigation.

miR-122 has recently been identified to promote HCV replication, and an antisense inhibition of miR-122 has been shown to inhibit HCV. miR-122 is also involved in cholesterol metabolism, and inhibition profoundly reduced serum cholesterol levels in chimpanzees. IL28B type has recently been associated with serum LDL levels in CHC patients. It was therefore plausible and important to investigate whether the IL28B polymorphism was associated with miR-122 expression levels. However, miR-122 was not observed to be associated with either IL28B type or ISG expression. Furthermore, in the treatment subgroup, we observed a significant relationship between miR-122 levels and treatment outcome, that was independent of IL28B genotype. This suggests that these two pathways may act independently to influence response to IFN-based treatment of HCV infection. The finding suggests that future treatment modalities for HCV infection may potentially take advantage of both IFN-dependent and miR-122-dependent modes of viral eradication, which may produce a synergistic effect on viral clearance.

The current study tests two of the most obvious hypotheses regarding the mechanism for the effect of IL28B genotype on HCV clearance: 1) differential intrahepatic expression of IL28B mRNA based on IL28B genotype, and 2) differential antiviral potency between protein sequence variants of the IL28B gene product, IFN-α. The relationship between IL28B genotype and IL28B mRNA in the literature has been controversial. Previous studies have suggested that the effect of IL28B genotype on HCV treatment response may occur through differential expression of IL28B by genotype, with the low-response genotype showing lower expression of IL28 in whole blood from healthy volunteers, or whole blood from HCV infected patients. More recently, Honda and colleagues showed that expression levels were similar in HCV-infected livers according to IL28B -type. Here, we also show, using a highly specific assay for IL28B mRNA, that IL28B genotype is not associated with IL28B expression in liver biopsies from HCV infected patients. This suggested that the biological effect of the IL28B polymorphism might be to effect potency, which would require a nonsynonymous causal variant. Previous work has suggested a possible causal role for a polymorphism in exon 2 (rs8103142, Arg70Lys). However, we rigorously investigated the potential for Arg70Lys to impact antiviral potency of IL28B and found no discernible difference between variants. As fine mapping studies have not demonstrated any other coding variants to be highly linked with the discovery SNP, this suggests that the mechanism likely involves an explanation other than signaling potency. Chen and colleagues have recently shown that ISG expression levels may differ between different cell populations in the liver, where ISG up-regulation was more pronounced in hepatocytes in nonresponders to IFN therapy, and in Kupffer cells in responders. As microarray analysis does not differentiate the cell of origin of the observed ISG expression, it is possible that the IL28B polymorphism has cell-specific effects on expression that may not have been detected in the current study. Alternatively, genotype-specific polymorphisms could conceivably impact post-transcriptional regulation of IL28B expression in liver (i.e. mRNA splicing, translation, etc.) that would be missed by microarray and RT-PCR analyses.

The results presented here provide a paradox. The non-CC genotypes, associated with higher level ISG expression in the setting of chronic hepatitis C infection, have previously been associated with lower rates of spontaneous clearance. In contrast, the CC genotypes, in which low level ISG expression is observed, have been associated with high rates of spontaneous clearance. It may be that the differential pattern of gene expression is specific to chronic HCV, and cannot be extrapolated to acute HCV. Alternatively, a more integrative hypothesis is that the effect of the poor-response IL28B variant is to ‘sabotage’ the liver’s response to type 1 IFN - perhaps by exhausting the common signaling pathway. In contrast, in the setting...
of the good-response CC variant, the liver remains relatively dormant, and ‘primed’ for type 1 IFN response. However, this hypothesis is speculative given current knowledge. Little is known about the interaction between type 1 and type 3 IFNs in vivo. Cell culture models of HCV replication suggest an additive antiviral effect of IFN-α and IFN-α/β, but no data is available yet for IFN-β3; furthermore, these experimental models do not account for the host immune milieu. The precise pattern of interaction between type 1 IFN and type 3 IFN signaling therefore remains unclear.

In conclusion, these data provide novel insights into the host genetic regulation of IFN signaling in the livers of patients chronically infected with HCV. The I2L28B polymorphism is associated with differential intrahepatic gene expression profiles, where the good response I2L28B variants are associated with lower levels of hepatic ISG expression. I2L28B genotype was not associated with differences in intrahepatic I2L28B gene expression, and protein sequence variants of IFN-α2 do not appear to explain the differences in ISG expression or anti-HCV response by I2L28B genotype. Thus, the biological mechanism underlying the influence of the I2L28B polymorphism on IFN treatment response remains unclear. Further examination of the noncoding polymorphisms in the I2L28B gene region, and of the dynamics of type 1 IFN sensitivity in the setting of sustained IFN-α activity, both in vitro and in vivo, may provide a fuller understanding of the role of IFN-α in natural clearance of HCV and its pharmacological treatment.

References


