Pharmacogenetics of hepatitis C

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Recent discoveries have highlighted the influence of host genomics on hepatitis C virus (HCV) infection outcomes. As a result, our views on hepatitis C pathogenesis and therapeutic approaches have been transformed. The recognition of the impact of single-nucleotide polymorphisms (SNPs) of the genes interleukin 28B (IL28B), inosine triphosphatase (ITPA) and low-density lipoprotein cholesterol receptor (LDLR) may lead to refinements in the pharmacogenomic prediction of antiviral response and drug-related toxicities and favour the discovery of new therapeutic targets for hepatitis C. Although the relevance of host genetics may be less in the setting of very potent new direct-acting antivirals (DAAs), genetic markers may continue to aid decision making regarding the length of therapy. Moreover, in several populations, such as HIV/HCV-coinfected patients, current therapy with peginterferon-α/ribavirin will continue in use for most patients, and thus host factors will retain their predictive value for treatment outcomes for a while.

Keywords: hepatitis C virus, ribavirin, ITPA, IL28B, interferon

Introduction

Nearly 200 million people worldwide are chronically infected with the hepatitis C virus (HCV). In the absence of therapy, a quarter of these patients will go on to develop cirrhosis. The aim of treatment against HCV is eradication of the virus. This goal is presumed to have been reached when viraemia is no longer recognized 6 months following discontinuation of therapy. Unfortunately, this sustained virological response (SVR) is achieved in fewer than half of patients treated with peginterferon-α plus ribavirin (pegIFNα/RBV) and who have HCV genotype 1, which is the most prevalent worldwide.1,2 Treatment outcomes are worse in HIV/HCV-coinfected subjects,3 in whom interactions between HCV medications and antiretroviral agents may contribute to impaired virological responses.4 Given the wide variability in HCV treatment outcomes, prediction of SVR may help to individualize therapy and make rational decisions on the initiation of antiviral drugs.

A number of host and viral factors have been associated with SVR. Host factors include age, gender, liver fibrosis stage, metabolic abnormalities and race.1,2 Viral predictors of SVR include HCV genotype, plasma HCV RNA level and early viral kinetics on the initiation of antiviral drugs.1,2 However, even after considering host and viral factors, there is still a large amount of unexplained variability in treatment outcomes, suggesting that the genetic background modulates the likelihood of viral eradication when pegIFNα/RBV therapy is used. The human genome is composed of roughly 3 billion base pairs, although there are <25,000 protein-coding genes. Recent technological advances have allowed comparison of more than half a million genetic variants in individuals with disease and healthy controls.5,6 In genome-wide association studies (GWAS), several single-nucleotide polymorphisms (SNPs) have recently been shown to influence the natural history and treatment outcomes of hepatitis C. Although allelic variants of interleukin 28B (IL28B) are the most frequent, SNPs at other genes have also been shown to influence susceptibility to treatment or the risk of drug-related toxicities (Figure 1).

IL28B

Allelic variants of IL28B were first linked to the interferon treatment response in hepatitis C patients in late 2009. Three separate GWAS identified SNPs at chromosome 19 near the IL28B gene as strong predictors of treatment-induced clearance of HCV infection caused by HCV genotype 1.7–9 These results were soon confirmed by others testing distinct populations.10–13 Thereafter, IL28B allelic variants were also found to be strongly associated with spontaneous HCV clearance.14 Although several SNPs around the IL28B gene with strong association with SVR to pegIFNα/RBV were initially described, the best association has been established for rs12979860.15

IL28B and HCV immune responses

The precise mechanisms by which IL28B SNPs influence treatment responses are unclear. Interferon (IFN)-α3 is the protein transcribed by the IL28B gene. The synthesis of this cytokine, which has antiviral activity, is induced upon viral infection. Like IFNα, IFNα stimulates the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway, which eventually prompts the expression of IFN-stimulated genes (ISGs), which results in antiviral activity against HCV.16 Although abnormal
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- IL28B
- ITPA
- LDL-cholesterol receptor
- Others: IP-10, HCV core mutations 70/91, etc.

Figure 1. Main genetic determinants of hepatitis C natural history and treatment response.

expression of the IL28B (IFNL3) gene could theoretically enhance or reduce antiviral activity, remarkably the intrahepatic expression of IL28B does not differ across IL28B genotypes. However, the expression of intrahepatic ISGs is strongly associated with IL28B alleles, and increased expression of ISGs is associated with reduced response to exogenous IFNα. Accordingly, unfavourable IL28B genotypes have been linked to up-regulated intrahepatic expression of ISGs.

Shortly after starting pegIFNα/RBV therapy, a significant increase in plasma IFNα3 levels is noticed in patients with favourable IL28B alleles, but is not seen in patients with other variants. Therefore, the underlying mechanism mediating the effect of IL28B SNPs on IFNα-induced HCV clearance might involve changes in ISG expression, leading to distinct levels of endogenous IFNα3 secretion in response to exogenous IFNα.

IL28B and spontaneous HCV clearance

The rs12979860 CC genotype is strongly associated with natural clearance of HCV. This link has been confirmed in different scenarios, such as children with vertically transmitted HCV infection; women infected with HCV through contaminated anti-D blood products; and adults with acute hepatitis C, establishment of chronicity occurs less frequently in those infected with HCV genotype 1 or 4 who harbour favourable IL28B allelic variants. It is noteworthy that this protective effect is not seen in infections caused by HCV genotype 2 or 3. In a recent study conducted in HCV-infected individuals with an episode of acute hepatitis C, rs12979860 alleles did not influence susceptibility to HCV infection caused by distinct HCV genotypes, with the exception of CC carriers exposed to HCV-1/4, who progressed less frequently to chronicity.

IL28B and viral load

Besides their effect on spontaneous viral clearance, IL28B alleles have been associated with plasma HCV RNA levels. This was the case in the pivotal study conducted by Ge et al. The favourable treatment outcome of IL28B CC carriers was significant even in the presence of a high baseline viral load (HCV RNA >600 000 IU/mL). However, this link between the favourable rs12989760 CC genotype and a well-defined predictor of a lack of SVR, such as high HCV RNA, follows an opposite direction to that what would be expected and is therefore counterintuitive. However, the same association has been noticed previously, including a study conducted in HIV/HCV-coinfected patients. Hypothetically, low baseline expression of ISGs linked to favourable IL28B alleles might result in lower spontaneous antiviral activity in the host, allowing enhanced HCV genotype 1 replication. It should be acknowledged that other studies have not confirmed the association between IL28B genotypes and plasma HCV RNA and therefore it has to be viewed as controversial.

IL28B and progression to cirrhosis

The lower expression of ISGs in patients with favourable IL28B alleles might lead to poorer control of HCV genotype 1 replication and, in turn, perhaps to more severe liver damage. Some authors have shown associations of favourable IL28B variants with enhanced liver inflammation and fibrosis and with fibrosis progression. This has not been confirmed by others, although the latest study excluded patients with decompensated cirrhosis and black ethnicity, which could have influenced the results. Further studies should be undertaken to confirm the association between liver fibrosis/inflammation and IL28B allelic variants. Figure 2 summarizes the postulated broader effects of IL28B variants on the natural history of HCV.

IL28B and treatment outcome

In the pivotal study by Ge et al., seven SNPs were associated with SVR after correction for multiple comparisons. The SNP most strongly linked to SVR was rs12979860. Other SNPs were highly correlated with rs12989760 due to linkage disequilibrium. Of note, all these SNPs were located in the IL28B region on chromosome 19. Tanaka et al. found other SNPs linked with treatment failure in an independent GWAS, the strongest being with rs8099917. However, this study did not test the SNP reported by Ge et al. (rs12989760). In a third independent GWAS, Suppiah et al. also showed an association between rs8099917 and SVR. Subsequently, Rauch et al. found other SNPs near the IL28B locus to be strongly associated with the response to pegIFNα/RBV therapy. The current consensus is that, globally, the SNP with the strongest effect on SVR is
IL28B variants and outcome in liver transplantation

The response to hepatitis C treatment is significantly higher in recipients with favourable IL28B variants compared to those with IL28B non-VAR variants. This is particularly true for liver transplantation outcomes. However, the impact of these variants on the response to antiviral therapy is more pronounced in patients with chronic hepatitis C, with no significant impact on treatment outcomes in patients with acute hepatitis C. The predictive value of IL28B for SVR in the treatment of acute hepatitis C has been extensively investigated. German authors evaluated the effect of IL28B variants on treatment outcomes in patients with acute hepatitis C and found that the rs12979860 CC genotype was associated with higher SVR rates in the subset of HCV-3 patients that did not experience rapid viral decline. In HIV/C Hepatitis C-coinfected patients, rs12979860 CC has been associated with viral decline during the first 4 weeks of therapy and the rs12989760 CC genotype was strongly associated with very early viral decline during the first phase of viral kinetics throughout treatment. The rs12979860 CC genotype was also found across all HCV genotypes 2/3 carriers. In contrast, individuals infected with HCV genotypes 1/4 exhibited a lower SVR rate in patients with favourable IL28B genotypes. IL28B variants are strong determinants of HCV RNA kinetics and outcome in the subset of patients without RVR. The available information suggests that the impact of IL28B variants on SVR in the treatment of acute hepatitis C is lower in patients infected with HCV genotypes 3/4, than in patients infected with HCV genotypes 1/2. However, the effect of IL28B variants on SVR in the treatment of chronic hepatitis C is limited to treatment outcomes in patients infected with HCV genotypes 1/4, with no recognition of any significant impact on early viral kinetics.

Specific analysis of results in HCV genotype 3 patients in more recent studies has concluded that there is no differential effect of IL28B genotypes 1/4 with no recognition of any significant impact on early viral kinetics. The rapid elimination of viral particles during the first hours/days of treatment is assumed to result from blocking the production or release of virions, and the effect of IL28B variants was also seen across all HCV genotypes 3/4. In contrast, individuals infected with HCV genotypes 1/4 exhibited a lower SVR rate in patients with favourable IL28B genotypes. The available information suggests that the impact of IL28B variants on SVR in the treatment of acute hepatitis C is lower in patients infected with HCV genotypes 3/4, than in patients infected with HCV genotypes 1/2. However, the effect of IL28B variants on SVR in the treatment of chronic hepatitis C is limited to treatment outcomes in patients infected with HCV genotypes 1/4, with no recognition of any significant impact on early viral kinetics.

Table 1. Impact of IL28B variants on response to pegIFNα/RBV therapy in chronic hepatitis C in several studies

<table>
<thead>
<tr>
<th>Genetic testing method (SNPs)</th>
<th>HIV coinfection</th>
<th>Number of patients</th>
<th>Ancestry</th>
<th>HCV genotypes SVR</th>
<th>Genotypes SVR</th>
<th>Allele-specific PCR</th>
<th>Allele-specific PCR</th>
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<tr>
<td>Ge et al. (rs12979860)</td>
<td>no</td>
<td>1137</td>
<td>Caucasian, African-American and Hispanic</td>
<td>1</td>
<td>1</td>
<td>yes</td>
<td>yes</td>
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<td>Tanaka et al. (rs8099917)</td>
<td>no</td>
<td>142</td>
<td>Japanese</td>
<td>1</td>
<td>1</td>
<td>yes</td>
<td>yes</td>
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<tr>
<td>Suppiah et al. (rs8105790)</td>
<td>yes</td>
<td>293</td>
<td>Caucasian</td>
<td>1</td>
<td>1</td>
<td>yes</td>
<td>yes</td>
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<tr>
<td>Rauch et al. (rs8099917)</td>
<td>no</td>
<td>1015</td>
<td>Caucasian</td>
<td>1, 2, 3 and 4</td>
<td>G, 63%; non-G, 28%</td>
<td>1, 2 and 3</td>
<td>CC, 68%; non-CC, 28%</td>
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<tr>
<td>McCarthy et al. (rs12979860)</td>
<td>yes</td>
<td>231</td>
<td>Caucasian and African-American</td>
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<td>1, 2, 3 and 4</td>
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<tr>
<td>Rallón et al. (rs12979860)</td>
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<td>196</td>
<td>Caucasian</td>
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<td>CC, 85%; non-CC, 67%</td>
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<tr>
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<td>154</td>
<td>Caucasian</td>
<td>1, 2, 3 and 4</td>
<td>CC, 93%; non-CC, 77%</td>
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<td></td>
</tr>
</tbody>
</table>

HCV mono-infection (n=779) and HIV co-infection (n=236).
Replication cohort (n=172).
Replication cohort (n=555).
For rs8099917 gene.
recipient and donor IL28B variants. However, characterization of donor IL28B variants may be subject to error when it has not been done in advance, as recipient cell infiltration of liver grafts occurs soon after transplantation.

**IL28B variants and response to new direct-acting antivirals**

The effect of IL28B variants on the likelihood of SVR in patients receiving the new direct-acting antivirals (DAAs) against HCV genotype 1 is less strong than in those treated with pegIFNα/RBV alone, since drugs with greater antiviral potency make host factors less relevant. However, some particular aspects, such as the value of IL28B variants in defining therapy duration, may require further attention. IL28B variants have been reported to predict SVR in treatment-naive, HCV genotype 1 patients that start boceprevir-based therapy (Figure 3). Conversely, CC and non-CC rs12979860 genotypes were associated with similar rates of SVR in pretreated patients receiving pegIFNα/RBV along with boceprevir. However, when the response to pegIFNα/RBV during the lead-in phase was taken into account, IL28B variants no longer predicted SVR independently.

In IFNα-naive patients that initiated telaprevir-based combinations, IL28B variants predicted SVR rates (Figure 3). For IFNα-experienced patients that received telaprevir, the modality of prior response is the major predictor of SVR, with minimal or null impact of IL28B variants. Only with very potent DAAs, such as the HCV protease inhibitor sinemevir (TMC-435), may the influence of IL28B variants on SVR become negligible, as it is overcome by the strong antiviral effect of the drug.

**Integration of IL28B testing in pegIFNα/RBV treatment prediction**

Although IL28B variants are among the strongest predictors of the response to pegIFNα/RBV in chronic hepatitis C, IL28B characterization is not able to predict SVR in all treated patients. Thus, it is worth considering testing for IL28B variants together with other parameters to enhance the accurate prediction of SVR. Different models have recently been built that incorporate IL28B information to predict SVR. The Prometheus index (www.fundacionies.com/prometheusindex.php) was originally developed in HIV/HCV-coinfected patients and more recently has been validated in HCV-monoinfected individuals. It considers four baseline variables: HCV genotype, serum HCV RNA levels, liver fibrosis staging as measured by transient elastography, and rs12979860 genotype. The performance of this index has been shown to be very good, with an area under the curve for SVR of 89%. Besides including the genetic information from IL28B, the Prometheus index has the advantage of considering
liver fibrosis staging based on information provided by elastometry, a non-invasive tool; thus, it can be used in patients who do not have a recent liver biopsy. The 2011 European guidelines for HIV/AIDS (www.europeanaidsclinicalsociety.org) have adopted the Prometheus index, recommending it as useful tool to drive appropriate treatment decisions in hepatitis C patients.46

Inosine triphosphatase (ITPA)

Ribavirin-induced haemolytic anaemia can complicate the management of patients treated for hepatitis C. Up to 15% of patients have to reduce ribavirin dosing due to severe anaemia. Two SNPs at the ITPA gene, known to be functionally responsible for ITPA deficiency, have been shown to be associated with the risk of ribavirin-induced anaemia.47 The mechanisms by which ITPA deficiency protects against ribavirin-induced decline in haemoglobin levels are unclear, but may involve inhibitory competition between inosine and ribavirin triphosphate forms48 (Figure 4). These findings have been reproduced in HIV/HCV-coinfected patients treated with pegIFNα/RBV.49,50 In this population, the decrease in haemoglobin is generally more pronounced and the need for erythropoietin and ribavirin dose reductions is more frequent than in HCV-monoinfected individuals, and information on ITPA allelic variants is therefore even more relevant. It is noteworthy that ITPA polymorphism does not predict SVR70,48 and its role is therefore limited to the prevention of toxicity. Thus, ITPA testing is unlikely to be of value in driving any significant treatment decision in the new era of DAAs, as ribavirin exposure will progressively lose its importance, to be replaced by new, specific antivirals. In the meantime, routine haemoglobin monitoring will remain key when making decisions regarding erythropoietin use and/or ribavirin dose reductions.

Low-density lipoprotein-cholesterol receptor

A genetic variant of the low-density lipoprotein (LDL)-cholesterol receptor gene (LDLR) has recently been shown to predict SVR to pegIFNα/RBV therapy in chronic hepatitis C patients.51 This finding has recently been extended to HIV/HCV-coinfected individuals.52 In HIV/HCV coinfection, higher levels of LDL-cholesterol are found in rs12979860 CC than in non-CC carriers.13 This effect might be due to reduced LDLR gene expression induced by lower activity of endogenous IFNα in CC than in CT/TT carriers.

As LDLR is a putative HCV receptor,53 both IL28B and LDLR allelic variants could interact to modulate HCV replication. Not surprisingly, consideration of both IL28B and LDLR genotypes improves the predictive value for SVR over the assessment of these variables singly.52

In HIV/HCV-coinfected patients harbouring HCV genotype 1 who are treated with pegIFNα/RBV, LDLR variants influence the risk of HCV relapse.52 This is in contrast with IL28B variants, which influence early viral kinetics29,30 but not the risk of HCV relapse upon discontinuation of therapy.11 Thus, the effect of both IL28B and LDLR variants is somewhat complementary in the prediction of SVR in chronic hepatitis C.

Other genetic determinants of hepatitis C treatment response

Lower baseline levels of IFNα-induced protein 10 (IP-10) predict the achievement of SVR with a high likelihood.54,55 When both IP-10 and IL28B allelic variants are considered together, the prediction of SVR can be refined and increases significantly. Mutations at codons 70 and 91 within the HCV core gene, which overlaps the IFN sensitivity-determining region, influence the response to pegIFNα/RBV therapy. Substitutions at amino acid 70 predicted SVR independently from IL28B SNPs in Japanese patients infected with HCV genotype 1.41,56 Thus, considering information from both IL28B testing and HCV core protein substitutions yields more accurate baseline prediction of SVR.

Individualization of hepatitis C therapy

The introduction of IL28B testing in routine clinical practice will guide therapeutic decisions in patients with chronic hepatitis C, particularly in those infected with HCV genotypes 1/4. Although the most robust data are limited to showing that IL28B variants influence the response to pegIFNα/RBV alone or in combination with DAAs, preliminary information suggests that even IFNα-sparing antiviral combinations for hepatitis C might perform differently according to IL28B allelic variants, as has been pointed out recently in the INFORM-1 trial.57 Only the use of very potent antiviral agents, such as simeprevir (TMC-435),43

Figure 4. ITPA gene polymorphisms and ribavirin-induced haemolytic anaemia. ITP, inosine triphosphatase; RBV-TP, ribavirin triphosphate.
might override the impact of IL28B variants on treatment outcomes, making it possible to disregard IL28B testing.

In HIV/HCV-coinfected patients, pegIFNα/RBV therapy will likely remain as the standard of care for some time, with a few special considerations with respect to HCV monoinfection. On the other hand, the efficacy of DAA-based regimens could be lower in coinfected individuals and therefore treatment outcome would probably be more influenced by host factors, including genetic traits. Moreover, for some antiretroviral regimens, the risk of drug–drug interactions may make difficult or even preclude the use of first-generation DAs. Likewise, infection with HCV genotypes other than 1, particularly HCV-4 in Western Europe, will not benefit from using first-generation DAs. In this scenario, standard therapy will remain in use for the foreseeable future, and the role of host factors (i.e. IL28B variants) in predicting treatment outcomes will remain important.

Therapy should not be deferred in patients with advanced liver fibrosis. In the absence of contraindications, therapy should be given to all those who are infected with HCV genotypes 2/3; likewise, treatment should be given to those with HCV genotypes 1/4 and favourable IL28B variants. Only when there is a high viral load and null or moderate liver fibrosis may HCV genotype 1 patients with unfavourable IL28B variants defer therapy and wait for the arrival of next-generation DAs. In the meantime, a reasonable option in these patients is to monitor the progression of liver fibrosis by non-invasive tools, to avoid any alcohol intake and to control harmful metabolic abnormalities while maintaining a normal body weight with diet and exercise.

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Transparency declarations
None to declare.

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