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Impact of host and virus genome variability on HCV replication and response to interferon

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Abstract

Since the discovery of hepatitis C virus (HCV), treatment has proven difficult and the regimen of pegylated interferon- and ribavirin is only effective for half of patients. Evidence suggests that host and viral genome variations play a role in either viral clearance or persistence. Powerful genomic technologies have made it possible to study genome-wide associations with treatment response, which yielded critical genetic polymorphisms that predict treatment response. This has important implications for treatment of HCV infection and opened the door to the possibility of genetic marker-guided treatment (personalized medicine). This review will focus on the recent advances in understanding host and viral genetic variations with regards to treatment and the importance for future therapeutic intervention.

Introduction

Hepatitis C virus (HCV) is a leading cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [1] and new estimates suggest a global prevalence of 2.35%, affecting approximately 160 million individuals [2]. Up to 80% of acutely infected individuals will develop chronic infection and as many as 5% eventually progress to liver cancer [3]. Treatment of HCV infection with pegylated interferon- and ribavirin (pegIFN-/RBV) is successful in only 50% of patients [4]. Additionally, ethnic differences in HCV clearance and response to treatment are quite striking suggesting genetic factors play a vital role [5,6]. New direct-acting antivirals (DAAs) have been introduced to the standard regimen and can increase the success to approximately 70% but may also lead to more adverse side effects [4,7,8].

HCV is an enveloped single-stranded RNA virus that targets hepatocytes in the liver. The HCV genomic RNA encodes for a large (~3000 amino acids) polyprotein, which is cleaved into structural and non-structural proteins by host and viral proteases. Like other RNA viruses, HCV replication is error-prone and has a high mutation rate. In addition to mutation, HCV infection produces a large population of new virions each day (10^{12-13}) because of a large reservoir of infected hepatocytes [9]. Thus, viral genetic diversity can play an important role in viral persistence and treatment response, although the precise determinants are still under investigation.

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In addition to viral genome variation, the host genomic variation is closely linked to success or failure during treatment. For this review we will focus on the identification of polymorphisms in the *interferon lambda 3* (*IFNL3*, also known as *IL28B*) gene that are important for viral clearance during treatment. We will also discuss the implications of these findings for the future of DAA-based HCV treatment.

Viral genetic diversity and treatment response

The HCV RNA-dependent RNA polymerase NS5B lacks a 3' to 5' exonuclease, which is vital for proofreading activity. Thus the low fidelity of this enzyme yields a high degree of genetic variations and the estimated mutation rate for HCV is on the order of 10^{-4} to 10^{-5} substitutions per nucleotide copied [10]. Thus, HCV exists in vivo as a quasispecies, which is a dynamic distribution of nonidentical but closely related genomes [11]. This has contributed to the large genetic diversity observed within the HCV-infected population. Indeed, HCV has six major genotypes that differ from each other by 30-35% at the nucleotide level and each genotype also has subtypes that may vary by 20-25% [12]. Importantly, treatment with pegIFN- α /RBV is 70-80% effective for patients infected with genotype 2 and 3, but is only 40-50% effective for genotype 1, which is the most prevalent genotype in the US [13]. This suggested that viral genome variability could play a role in determining treatment outcome. To date, variations in four HCV proteins, E2, NS3/4A, core, and NS5A, have been suggested to correlate with outcome of antiviral treatment. Due to space constraints we will limit our discussion to core and NS5A.

Core

The HCV core is an RNA-binding protein that forms the nucleocapsid of the virus. Core is important for viral assembly and is closely associated with lipid droplets [14,15]. Specific polymorphisms in the core protein (amino acids 70 and 91) are associated with better response to treatment of genotype 1 [16-19]. However, these same amino acid substitutions from genotype 4 infected patients did not correlate with treatment response [20]. A majority of the published data is from Japanese patients infected with genotype 1b, thus it is still unknown if these core polymorphisms can be attributed with treatment response in other populations. Additionally, in vitro studies have produced conflicting results. One report indicated no differences in IFN resistance between WT and core 70/91 mutants, while another study indicated a strong association with core mutants 70/91 and IFN resistance [21,22]. These contradictions may be in part be caused by the different methodologies used by each study. Nonetheless, there is some evidence that the core protein may directly antagonize the IFN response. Initial results demonstrated that overexpression of core inhibited STAT1 activation [23,24]. Recent studies report that core can block interferon regulatory factor 1 (IRF-1) expression, which leads to downregulation of IRF-1-dependent genes [25]. A second study identified a domain within core that antagonized interferon regulatory factor-3 activation possibly through inhibiting dimerization [26]. However, most of these studies use overexpression of core rather than natural infection with infection-competent HCVcc. Taken together this suggests a possible role for HCV core in attenuating host innate immunity, but more definitive evidence has to come from a more biologically relevant model.

NS5A

NS5A is a multifunctional phosphoprotein that is required for several stages of the virus life cycle [27]. Early studies suggested that variations or mutations in the C-terminal region of NS5A (amino acids 2209-2248), which was later designated as the interferon sensitivity-determining region (ISDR), were associated with better response to pegIFN- α /RBV [28]. Studies in Japanese patients confirmed that greater than four mutations within the ISDR

correlated with sustained virological response (SVR) to interferon treatment [29,30]. However, HCV 1b isolates from different geographic regions showed no correlation between mutations in ISDR and treatment response, thus the phenotype could not be solely explained by these genetic variations [31-33]. More recent studies have identified several other polymorphisms in different C-terminal region of NS5A (amino acids 2334-2379) termed the pegIFN- α /RBV resistance-determining region (IRRDR) [34-36]. Similar to the ISDR, as mutations accumulated within this region the response to treatment improved in patients infected with genotypes 1b and 4.

NS5A may also play a direct role in antagonizing the antiviral effects of IFN- α . Early studies in vitro indicated that NS5A ISDR was able to bind directly to the catalytic domain of the IFN-induced protein kinase PKR and repress its activity [37]. More recent studies have demonstrated that NS5A is able to bind to STAT1 and inhibit its phosphorylation [38-40]. Phosphorylated STAT1 binds to STAT2 and IFN-regulatory factor 9 to complex, which translocates to the nucleus to induce expression of IFN effector proteins. Similar to studies involving core, these studies observed these effects after overexpression of NS5A, which does not directly mimic natural HCV infection. In addition, a study using the HCV replicon system did not observe any contribution of NS5A to IFN resistance in cell culture [41]. Nonetheless, it is possible that NS5A and its sequence variations may contribute to HCV persistence during IFN treatment.

Discovery of *IFNL3* (*IL28B*) polymorphism

Previously observed differences in the HCV clearance and response to treatment among different ethnic groups had long suggested that host genetic factors might play an important role. Initial studies examined candidate genes to identify differences or single nucleotide polymorphisms (SNPs) between two populations. A SNP is base-pair variant within the genome that has a frequency greater than 1% [42]. Several SNPs were identified in genes that are involved in the innate defense against HCV including MxA, OAS1, EIF2AK2, IFNAR1, IL-6, MHC, and MAPKAPK3 [43-49].

Advances over the past decade have made it possible to perform genome wide association studies (GWAS) to detect SNP frequencies between two populations. For HCV, this led to comparisons between patients that attain SVR vs non-response or non-SVR to peginterferon/ribavirin therapy. SVR is defined as undetectable viremia 24 weeks after completion of treatment. The initial GWAS study reported an association between the *IFNL3* (*IL28B*) polymorphism and positive treatment outcome [50•]. In rather quick succession, three different GWAS studies were published [51-53•]. The design and major findings for each study is summarized in Table 1. The *IFNL3* locus encodes for the type III IFN- λ protein that is induced by viral infection and induces antiviral activity through both innate and adaptive immune pathways [54]. Type III IFNs bind to a unique receptor and transmits the intracellular signal through a complex of STAT1, STAT2, and IRF9 to drive expression of interferon stimulated genes (ISGs) [55]. The original paper published by Ge et al., identified the *rs12979860* SNP located upstream of the *IFNL3* gene. Patients with the homozygous allele CC were 2-3 times more likely to achieve SVR compared to those with the unfavorable alleles TT or CT. This SNP was confirmed in a different cohort of 231 patients infected with genotypes 1, 2, or 3 [56]. Shortly after the initial discovery, three separate studies identified a neighboring *rs8099917* SNP and demonstrated that the major allele TT was associated with better response to treatment (Table 1). These two SNPs are in strong linkage equilibrium and therefore difficult to discern which one is more important in association with treatment response. Taken together, these results suggested that *rs12979860* and *rs8099917* are strong predictors of SVR after treatment. Currently there are clinically

available tests to examine the *IFNL3* genotype to aid in predicting a patient's response to dual therapy prior to commencing.

Approximately 70-80% of HCV infected individual's fail to resolve acute infection and progress to chronic infection. The reason some patients clear the virus and others do not is quite perplexing, since studies have shown that patients infected with the same inoculum still differed in their ability to clear the virus naturally [6]. The initial GWAS study also discovered that the *rs12979860* SNP genotype was associated with spontaneous clearance of HCV infection [50]. Additionally, a separate study confirmed that the *rs8099917* TT was associated with spontaneous clearance [53]. This phenomenon was corroborated by several other groups, which implicate these two SNPs in natural clearance of HCV infection [57-59].

Mechanism of IFNL3 SNPs in HCV treatment and clearance

Despite the dramatic effects the *IFNL3* polymorphisms exert on treatment and clearance of HCV the precise mechanisms leading to this outcome are still unclear. It is well known that IFN- α induces the expression of a multitude of ISGs that establish an antiviral state within effected cells. It is logical to hypothesize that the unfavorable genotypes exhibit a weak or attenuated ISG induction. However, it was demonstrated that hepatic expression of ISGs was markedly higher in patients who did not respond to interferon-based therapy [60-62]. As expected, the same correlation was extended to those with unfavorable IFN- α genotype [63,64]. Furthermore, patients with the favorable genotype (*rs12979860* C/C) exhibited lower expression of several ISGs and had increased expression of genes that suppress the antiviral state [65,66]. There was no difference in hepatic ISG expression between favorable and unfavorable genotypes in the absence of HCV infection [67]. Overall these findings suggest that reduced ISG expression is not the underlying mechanism and does not explain why the *IFNL3* polymorphisms predict treatment outcome. In contrast, the prevailing hypothesis is that the increased ISG expression observed in the unfavorable genotype may decrease sensitivity to exogenous IFN- α therapy and this desensitization could lead to treatment failure [68]. In regards to natural clearance of the virus, several groups also showed that individuals with the favorable genotype have a higher spontaneous viral clearance rate in acute HCV infection [16,50,56,69], consistent with the important role of IFN- α in HCV infection.

New and exciting evidence has emerged that identified a variant upstream of *IFNL3*, which creates a new gene, *interferon lambda 4 (IFNL4)* [70]. In this report the authors isolate a dinucleotide polymorphism (*ss469415590*), which either creates (G) or interrupts (TT) the open reading frame of *IFNL4*. The G variant correlates with poor treatment response while the TT variant, which abrogates the *IFNL4* ORF, is associated with positive treatment response. Interestingly the identified *ss469415590* polymorphism is strongly linked with *rs12979860* and appears to be a superior predictor of treatment response in patients of African ancestry. The authors further demonstrate that the INFL4 protein is able to induce ISG expression in hepatoma cells, but it is unclear at this time how this protein could impair HCV clearance. Much more work is needed to understand the role of IFNL4 in immunity, but this finding may provide valuable biological information and perhaps yield a new strategy for treatment of HCV.

IFNL3 polymorphisms and triple drug therapy

The identification of *IFNL3* genotype was an important advance in understanding the varied response during pegIFN- α /RBV treatment. However, new direct acting antivirals (DAAs) were approved for treatment in early 2011 and it's logical to question the importance of *IFNL3* in new treatment regimens. Two first-generation protease inhibitors, boceprevir and

telaprevir, are used in combination with pegIFN- α /RBV as the standard treatment for HCV [4,71]. Currently, the amount of data available to address this question is incomplete but there is evidence to suggest a diminished role for *IFNL3* genotype in the new era of treatment. Results from the boceprevir phase III trial (SPRINT-2) demonstrated that individuals with the favorable CC genotype (*rs12979860*) had similar SVR with or without boceprevir (82% and 78% respectfully) [72••]. However, with the addition of boceprevir the rates of SVR increased significantly in patients with the unfavorable genotypes (59% for CT and 71% for TT, compared to 27% and 28% respectfully). The findings with triple therapy using telaprevir were generally similar to that of boceprevir [73]. Patients with the favorable CC genotype had an increased chance of SVR to 90% while the response of the unfavorable genotypes was approximately 70% (due to the nature of this retrospective analysis, no formal statistics were performed). Of note, two recent reports demonstrate that *IFNL3* polymorphisms are still predictive of treatment response with telaprevir in Japanese patients [74,75]. The association of IFN- α genotypes with response to the current DAA-containing triple therapy is probably because inclusion of interferon is still necessary to achieve the optimal response in this regimen. Taken together, the current limited data suggests that the utility of IL28B genotyping is diminished with the addition of a DAA. Currently the American Association for the Study of Liver Disease (AASLD) does not recommend *IFNL3* testing for all patients prior to triple therapy, but does state that testing may be considered to acquire additional information about treatment response or duration [76]. For now it appears that *IFNL3* testing may still provide information that will aid in evaluating patients treatment options.

Conclusion

HCV is a dynamic and exciting field of study. This relatively small virus has evolved innumerable ways to persist and establish a chronic infection within the liver. Viral genetic diversity has created a complex pathogen that is very difficult to treat and can actively subvert innate immunity. Additionally, we now understand how key genetic determinants within the host can have powerful effects on treatment and clearance of HCV. Expanding this research may yield novel aspects about immunity and could provide new ways to target and disrupt HCV replication.

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Highlights

- Viral genome variants in Core and NS5A determine treatment outcome
- Host polymorphisms impact response to IFN treatment
- Newly discovered IFNL4 yields new possibility for HCV treatment

Figure 1. Summary of viral and genetic host factors that correlate to treatment response in HCV infection

Upon HCV entry, host recognition of viral RNA induces production of interferon and interferon stimulated genes (ISGs). HCV genetic polymorphisms are important for attenuation of these responses especially within NS5A (ISDR/IRRDR) and core (aa 70/91). Both viral proteins can disrupt vital signaling pathways in the interferon response, such as JAK/STAT and IRF3. Furthermore, host genetic variation is also correlated with response and clearance of HCV, but the underlying mechanism remains unknown. The prevailing hypothesis is that the unfavorable IFNL3 SNPs (*rs12979860 TT* and *rs8099917 GG*) lead to desensitization to IFN and thus result in treatment failure. Also new evidence pinpoints another possibility with the discovery of *IFNL4* SNP and its effect on treatment. *IFNL4* (*ss469415590*) may act similarly to the previous SNPs and produce a dampening effect in the response to IFN.

Table 1

Summary of the initial GWAS studies

Study	Ge et al., 2009	Suppiah et al., 2009	Tanaka et al., 2009	Rauch et al., 2010
Size ^I	1671 no replication	293 and 555 replication cohort	142 and 172 replication cohort	465 no replication
Ethnicity	Caucasian/African/Hispanic	Caucasian	Japanese	Caucasian
Comparison	SVR vs non-SVR	SVR vs non-SVR	SVR vs non-SVR and SVR/TVR vs NVR	SVR vs non-SVR
HCV genotype	1	1	1	1, 2, 3, and 4
Allele identified	<i>rs12979860</i> C/C	<i>rs8099917</i> T/T	<i>rs8099917</i> and <i>rs12980275</i> C/C and T/T	<i>rs8099917</i> T/T

^I Indicates the total number of patients analyzed, including the number in the replicate cohort. SVR = sustained virologic response, TVR = transient virologic response, NVR = null virologic response