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Forum Minireview

Novel Findings for the Development of Drug Therapy for Various Liver Diseases:
Genetic Variation in *IL-28B* Is Associated With Response to the Therapy for Chronic Hepatitis C

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Abstract. Hepatitis C infection is a global health problem. Spontaneous viral clearance was observed in approximately 30% of individuals with acute infection. In the therapy using a combination of pegylated interferon-α and ribavirin, approximately 50% of chronic hepatitis C patients infected with high viremia of hepatitis C virus infection (HCV) genotype 1 reached a sustained viral response. These findings were strongly expected to reflect variations of the host genome. To reveal genetic effects against viral clearance or treatment response, four independent groups applied a genome-wide association study (GWAS) to HCV infection. These groups almost simultaneously reported a strong association of interleukin (IL)-28B polymorphisms with viral clearance or final decision of HCV therapy. The discovered single nucleotide polymorphisms (SNPs) also revealed the enigma that the viral clearance rate was dependent on ethnic type. The significant SNPs are useful for prediction prior to treatment because of the strong association with clinical outcome. In addition, the unexpected results revealed by GWAS could promote the development of a novel drug related to IL-28B. Herein, we present current understanding in regard to the relationship between host variations and clinical outcome of hepatitis C.

Keywords: hepatitis C virus, genome-wide association study, interleukin-28, interferon-λ, single nucleotide polymorphism, liver disease

1. Introduction

Chronic infection with hepatitis C virus (HCV) presents a significant health problem worldwide with approximately 3% of the world population, that is, more than 170 million people. Only 20% – 30% of HCV-infected individuals recover spontaneously. The remaining 70% – 80% going on to develop chronic infection have a significant risk for progressive liver fibrosis and subsequent liver cirrhosis (LC) and hepatocellular carcinomas (HCC) (1). Successful treatment of chronic hepatitis C would reduce the morbidity and mortality of patients because around 8% of patients progressing to LC will develop HCC annually (2).

Spontaneous clearance following acute infection occurs in some cases for reasons that remain unclear, and previous studies report that 50% – 85% of patients progress to chronicity. The relationship between race and spontaneous viral clearance following acute infection have been reported (3 – 6). These characteristics based on ethnic types would suggest the effect of a host genetic factor on HCV infection.

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2. HCV treatment and the response

The current standard of care for the HCV infections comprises pegylated interferon (PEG-IFN)-α 2a or 2b plus ribavirin (RBV). Successful treatment, termed "sustained virological response (SVR)", was defined by an HCV RNA negative after 6 months of completing therapy, whereas a transient viral response (TVR) was defined as a reappearance of HCV RNA in serum after treatment was discontinued in a patient who had undetectable HCV RNA during the therapy or on completion of the therapy (Fig. 1). A non-viral response (NVR) was defined as cases with detectable viremia after and during treatment. The standard therapy is effective in only 42% – 52% of patients with HCV genotype 1 in the US and Europe (7 – 9). A significant difference in response to PEG-IFN&RBV therapy between ethnicities were reported: the SVR achievement of African Americans was only approximately 20% – 28% compared to 40% – 52% in Caucasian patients with genotype 1 infection (10 – 12) and 57% vs. 82% for genotype 2/3 (13). The current therapies are limited by expensive, ineffectiveness in part of the patients, and numerous potentially severe side effects, which cause dose reduction and/or premature termination of treatment. Additionally, premature withdrawal from IFN-based therapy (14) was necessary for 10% – 14% of the patients, leading to failure of the HCV therapy.

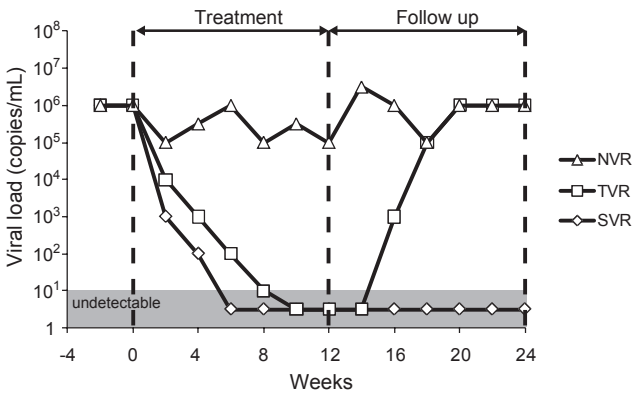

Fig. 1. Representative changes of HCV viral load in patients treated with PEG-IFN&RBV combination therapy. The response type under the therapy of PEG-IFN&RBV is divided into three groups: SVR, TVR, or NVR. SVR is defined as successful treatment, which is HCV RNA negative after 6 months of completing therapy. TVR is defined as a transiently negative for HCV RNA during treatment. However, after the end of therapy, HCV RNA of the patients reappears with impaired liver function in TVR. NVR is defined as a constitutive high viremia during and after treatment. SVR, sustained viral response; TVR, transient viral response; NVR, non-viral response

3. Viral factors associated with HCV therapy

To prevent treatment failure in such patients, we must identify the predictive factors leading to treatment failure as well as production of severe side effects in the clinic. Previous studies have reported that viral titer, mutations, or gene expression levels of innate immunity could be prediction factors for NVR using clinical specimens of chronic hepatitis C patients. Several viral factors such as genotype 1 (HCV-1), high baseline viral load, viral kinetics during treatment, and amino acid pattern in the IFN sensitivity–determining region have been found to be significantly associated with the outcome by a number of independent studies (15 – 17). Accumulated data have provided strong evidence that approximately 20% of patients with HCV genotype 1 have NVR to PEG-IFN&RBV. The reliable prediction for NVR would allow avoidance of side effects and reduce the cost of treatment in the 20% of patients with HCV-1 before starting the treatment.

4. Host factors associated with response to PEG-IFN&RBV therapy

Several host factors related to viral clearance have been reported based on clinical features or laboratory data, for example, gender, age <40 years, low HCV RNA level prior to treatment, lack of liver cirrhosis, and HCV genotypes 2/3 (18, 19). As for host genetic factor, candidate gene approaches have been adopted to identify host factors related to clinical outcomes, single nucleotides polymorphisms (SNPs), copy number variation (CNV), or insertion/deletion of genes. The approach could latently find weak associations and show significant differences because only one or a limited number of SNPs or gene loci are detected in candidate genes. The focused approach, however, contains the restraint to detect crucial factors. In detail, the selection of candidate regions for genetic study depends on the researcher's knowledge or the present data of the gene pathway.

In contrast, a recent genome-wide association study (GWAS) approach using high-throughput genotyping technology usually for SNPs, ranging from 300,000 to 900,000 SNPs in each sample, is able to detect strong association factors affecting disease susceptibility and drug response without any a-priori hypotheses on causative SNPs apart from the hypotheses (20, 21). On the basis of the GWAS, four independent groups assessed the role of genetic variation on response to PEG-IFN&RBV combination therapy for chronic hepatitis C patients, and the data was reported in a short-term (21 – 24). In all cases, the conclusive finding was that polymorphisms in or near the *IL-28B* gene strongly de-

termined the outcome of HCV therapy.

5. Study design of four studies for GWAS

Ge et al. and Suppiah et al. studied genetic variants associated with SVR to PEG-IFN&RBV therapy in individuals infected with HCV genotype 1 (21, 22). The former examined genetic factors associated with treatment response in patients from the IDEAL trial (Individualized Dosing Efficacy vs. flat dosing to Assess optimaL pegylated interferon therapy) (25), a large randomized controlled trial involving Caucasian, American-African, and Hispanic individuals in North America (n = 1137) (Table 1). The latter study group analyzed Caucasians consisting of 293 Australian individuals (Northern European ancestry) with HCV genotype 1 and also validates an independent replication cohort consisting of 555 Europeans from the UK, Germany, Italy, and Australia. These two study groups mainly investigated GWAS in Caucasians and analyzed host factors associated with SVR.

Tanaka et al. studied host factors associated with the response to PEG-IFN&RBV treatment in 142 Japanese patients with chronic hepatitis C of HCV genotype 1 for GWAS and prepared an independent replication cohort of 172 Japanese (Table 1) (24). In this study, patients were divided into three groups, SVR, TVR, or NVR. NVR vs. virological responder (VR) consisting of SVR and TVR was used for the predication of NVR factors. The data set of SVR vs. non-SVR (TVR and NVR) was constructed to discover the host factor related to SVR (Fig. 1).

Rauch et al. investigated 465 Caucasians infected with HCV genotypes 1, 2, 3, or 4 to reveal genetic variations associated with response to the combination therapy (23). A case control study was designed to detect genetic variations related to SVR in European individuals. Three study groups, except Suppiah et al., selected patients receiving at least 80% of the recommended treatment dose to emphasize genetic associations.

6. Identification of strongly significant SNPs associated with PEG-IFN&RBV therapy

Ge et al. identified a genetic polymorphism (rs12979860) near the *IL-28B* gene on chromosome 19, also known as IFN-λ3 (Fig. 2). Individuals with the CC genotype showed the association with an approximately two-fold change in response to PEG-IFN&RBV treatment compared with those with the TT genotype, both among patients of European ancestry ($P = 1.06 \times 10^{-25}$) and African-Americans ($P = 2.06 \times 10^{-3}$). An important finding in the study is the strong correlation between being a carrier of this SNP and SVR rates in diverse ethnic groups, which is significantly more frequent in European-Americans and Asian populations than in African-Americans. Approximately 23% – 55% of Africans (<40% of African-Americans) carry advantageous C-allele frequency of rs12979860, compared with approximately 53% – 85% of Europeans (<70% of European-

Table 1. Four GWAS groups studying host factor related to the response to HCV therapy

Study (Ref. No.)	Ge et al. (22)	Suppiah et al. (21)	Tanaka et al. (24)	Rauch et al. (23)
Region	Northern America	Northern Europe, Australia	Japan	Switzerland
Ancestry	Caucasian/ African/ Hispanic	Caucasian	Japanese	Caucasian
GWAS size	871/ 191/ 75	293	142	465
Replication	No replication	555	172	No replication
Case/ control	SVR vs. non-SVR	SVR vs. non-SVR	SVR vs. non-SVR SVR&TVR vs. NVR	SVR vs. non-SVR
Adherence	Over 80% adherent to PEG-IFN&RBV during the first 12 weeks of therapy	Not controlled	Over 80% adherent to PEG-IFN&RBV during the first 12 weeks of therapy	Over 80% adherent to PEG-IFN&RBV during the first 12 weeks of therapy
HCV genotype	1	1	1	1, 2, 3, 4
Significant SNPs	rs12979860	rs8099917	rs8099917	rs8099917
P value	1.37×10^{-28}	9.25×10^{-9}	1.18×10^{-18}*	3.11×10^{-8}
OR (95% CI)	3.1 (2.1 – 4.7)	1.98 (1.57 – 2.52)	12.1 (6.5 – 22.4)*	5.19 (2.9 – 9.3)
Platform	Illumina610-quad	IlluminaCNV370-quad	Affymetrix SNPs 6.0	Illumina Human 1M-duo, Human Hap550/ Human610W-quad

*The combined value in the study in comparison with SVR vs. non-SVR.

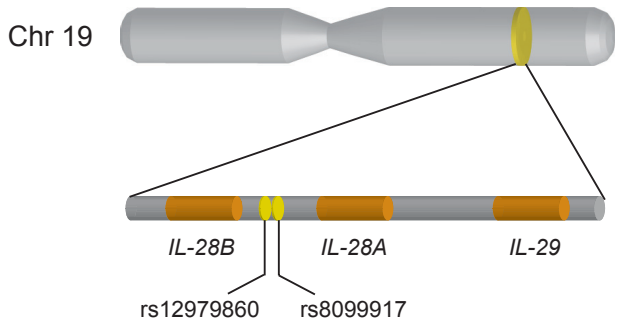

Fig. 2. *IL-28B* gene and SNPs location in chromosome 19. Four independent GWAS discovered SNPs strongly associated with the response to PEG-IFN&RBV therapy around *IL-28B* in chromosome 19. Ge et al. (22) reported rs12979860 as a strongly associated SNP, whereas Suppiah et al. (21), Tanaka et al. (24), and Rauch et al. (23) detected a statistical difference on rs8099917. These 2 SNPs are in strong linkage disequilibrium because these loci are very near to each other. The interferon lambda family consists of *IL-29* (IFN-λ1), *IL-28A* (IFN-λ2), and *IL-28B* (IFN-λ3), which are induced by type I IFN, or bacterial and viral infection.

Americans) and approximately 90% of Chinese and Japanese. Ge et al. showed that the SVR rates across different population groups displayed a striking concordance with the C-allele frequency. This SNP explained about half of the difference in response rates between African-Americans and Europeans.

Suppiah et al. and Tanaka et al. revealed the strong association of particular haplotypes of SNPs around *IL-28B* in the population of PEG-IFN&RBV therapy. The most significant SNPs in both study groups was rs8099917 (8 kb upstream of *IL-28B*) associated with SVR in European and Japanese patients (Fig. 2). Suppiah et al. also identified the association of rs8099917 in European ancestry with HCV genotype 1 based on the determination of SVR factors (combined $P = 9.25 \times 10^{-9}$, OR = 1.98, 95% CI = 1.57 – 2.52) (21). Homozygotes for the risk allele (rs8099917 G-allele) showed 2-fold higher risk of treatment failure than that of major allele homozygotes. In the gene expression assay, the minor allele of rs8099917 tended to suppress mRNA levels of *IL-28A/B*.

Tanaka et al. identified several SNPs significantly associated with NVR to PEG-IFN&RBV therapy in the GWAS and the replication study. All significant SNPs were located near the *IL-28B* locus on chromosome 19. The SNPs, rs12980275 or rs8099917, validated in an independent replication cohort showed the strongest association (combined $P = 2.84 \times 10^{-27}$ and 2.68×10^{-32}; OR = 17.7, 95% CI = 10.0 – 31.3; OR = 27.1, 95% CI = 14.6 – 50.3, respectively) (24). Interestingly, the minor alleles of the SNPs were accumulated in NVR (minor allele frequency of NVR = 74.3% for rs12980275 and 75.0% for rs8099917). Multivariate analyses containing genetic and clinical factors revealed that rs8099917 was the strongest predictor for response to therapy ($P = 0.0001$, OR = 37.68, 95% CI = 16.71 – 83.85).

The fourth GWAS was published on the response to HCV therapy, Rauch et al. studied patients infected with HCV genotype 1, 2, 3, or 4 (23). Rauch et al. also identified several SNPs around the *IL-28B* gene on chromosome 19 (Fig. 2). The strongest association with treatment failure was found with rs8099917 ($P = 3.11 \times 10^{-8}$, OR = 5.19). Interestingly, rs8099917 did not associate with the response to PEG-IFN&RBV therapy in genotype 2 or 3 patients. The contribution of host factors to genotype 2 or 3 clearance would be low because HCV genotype 2 or 3 is likely to be eliminated by the standard therapy compared with genotype 1. In individuals infected with HCV genotypes 1 and 4, the SVR rate of the patients harboring the minor allele was 28%, whereas that of the major allele homozygotes reached 63%. However, patients infected with genotypes 2 or 3 showed high viral response rate, approximately 80%, without statistical significance between the patients and the control.

7. The influence of genetic background on the statistical analysis

For the prediction of SVR, OR of the Japanese population was much higher than that of the other populations (Table 1). Individuals harboring the risk allele of rs8099917 or rs12979860 was approximately 10% in Asia, whereas the risk allele frequency was generally over 20% in European Caucasians. Moreover, individuals with the risk allele were the major population in individuals with African ancestry. The differences of allele frequency might explain, in part, the observed discrepancy in the response rate of viral clearance and the statistical power between racial groups.

Tanaka et al. extracted the data of TVR patients to analyze the genetic background. The minor allele frequency (MAF) of the strongly associated SNPs (rs8099917, located in the intergenic region between *IL-28A* and *IL-28B*) in TVR was similar to that of the SVR population (Fig. 3) (24). The statistical analysis for SVR prediction (SVR vs. non-SVR) using the SNPs showed lower statistical power (OR = 12.1) than that of NVR prediction (NVR vs. SVR plus TVR, OR = 27.1), indicating that the significant SNPs are strongly associated with the outcome of NVR. In other words, TVR patients share similar genetic background with SVR patients, and they would achieve SVR by prolonged therapy or PEG-IFN&RBV plus protease inhibitor.

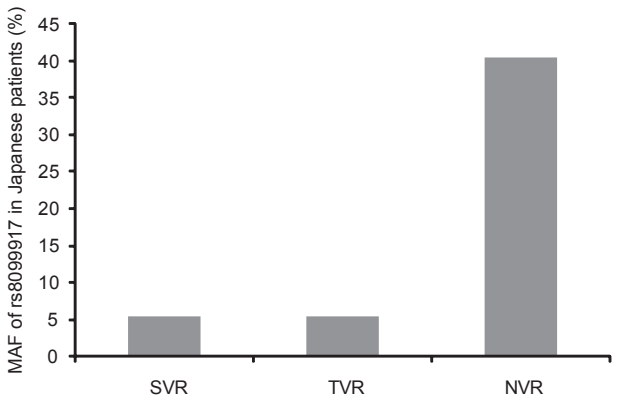

Fig. 3. Minor allele frequencies (MAF) of rs8099917 in each response type of chronic hepatitis C patients reported by Tanaka et al. (24). In the Asian population, the MAF of rs8099917 is approximately 10% according to Thomas et al. (20) and the dbSNPs international database. The MAF of chronic hepatitis C patients under a combination therapy of PEG-IFN&RBV revealed the deviation from that of the general population. The minor allele of rs8099917 accumulated in the population of NVR (approximately 40%), whereas those of SVR and TVR were occurred at lower frequency than those of the general population (approximately 5%).

8. SNPs associated with spontaneous clearance of HCV

Two study groups searched for common SNPs related to spontaneous elimination of HCV using a Caucasian cohort. Thomas et al. performed a candidate gene study on the rs12979860 SNP reported by Ge et al. to determine whether the SNP was also associated with spontaneous clearance of HCV infection (20). This study included 388 individuals with spontaneous HCV clearance and 620 with persistent HCV infection in a cohort consisting of HCV and HIV/HCV co-infected patients. The strong association of rs12979860 with spontaneous recovery was found in European and African American individuals (OR = 2.6, 95% CI = 1.9 – 3.8; OR = 3.1, 95% CI = 1.7 – 5.8, respectively) (Table 2). The association was also independent of co-infection with HIV, type of HCV transmission, and history of HBV infection.

Rauch et al. revealed the host factor associated with spontaneous clearance of HCV based on GWAS technology mounting more than 500K SNPs (23). The case-control study was designed for 347 individuals with spontaneous HCV clearance, 567 individuals with chronic hepatitis C, and 448 individuals with HCV/HIV co-infection. The significant SNP was also rs8099917 (combined $P = 6.07 \times 10^{-9}$, OR = 2.31, 95% CI = 1.74 – 3.04). The effect of HIV co-infection was also similar to that of HCV mono-infection ($P = 8.25 \times 10^{-5}$, OR = 2.16, 95% CI = 1.47 – 3.18; $P = 1.96 \times 10^{-5}$, OR = 2.49, 95% CI = 1.64 – 3.79, respectively) compared to Thomas et al. Note that rs8099917 was in strong linkage disequilibrium with rs12979860 in European and Asian individuals (26). These reports described by Thomas et al. and Rauch et al. seem to lead crucially identical results.

9. The characteristics of *IL-28B* and the IFN-*λ* family

IL-28B, referred to as IFN-*λ*3, belongs to the IFN-*λ* family, which consists of *IL-29*/IFN-*λ*1, *IL-28A*/IFN-*λ*2, and *IL-28B*. The *IL-28B* gene has been recently discovered and classified into type III IFN, a member of the class II cytokine family. This class II family includes type I, II, and III IFN and the *IL-10* family (*IL-10*, *IL-19*, *IL-20*, *IL-22*, *IL-24*, *IL-26*, *IL-28,* and *IL-29*). Peripheral blood mononuclear cells (PBMCs) and dendritic cells are main sources of IFN-*λ* (27, 28), which is induced by IFN-*α*, viral infection, and/or stimulations of toll-like receptors. IFN-*λ* behave as a interferon stimulated gene (ISG) of IFN-*α*, which is expressed at low levels by a broad variety of human cells, similar to IFN-*α* (29).

The signal pathway of IFN-*λ* is initiated through a membrane receptor distinct from that of type I IFN. The receptor is composed of heterodimer molecules consisting of an IL-28RA/IFN-*λ*R1 subunit and IL-10R2 subunit (27, 28). The IL-10R2 subunit is expressed broadly and shared by IL-10, IL-22, IL-26, and IFN-*λ*. Compared with the IFN-*α* /-*β* receptor, which is ubiquitously expressed, the IL-28RA receptor has a more restricted distribution. The signal transduction of IFN-*λ* receptor is mediated via Jak1 and Tyk2, which can induce the phos-

Table 2. Summary of associated SNPs regarding spontaneous clearance of HCV

Study (Ref. No.)	Thomas et al. (20)		Rauch et al. (23)		
SNPs	rs12979860		rs8099917		
Population	European	African	HCV mono-infection	HCV/HIV co-infection	Combined
P value	1.0×10^{-7}	1.0×10^{-4}	1.96×10^{-5}	8.25×10^{-5}	6.07×10^{-9}
OR	2.6	3.1	2.49	2.16	2.31
95% CI	1.85 – 3.84	1.75 – 5.88	1.64 – 3.79	1.47 – 3.18	1.74 – 3.04

phorylation of STAT1 and STAT2 molecules and is followed by the expression of ISG (30).

10. The antiviral effect of IFN-λ against HCV in basic studies or clinical trials

Antiviral effects of IFN-λs against HCV have been reported before the discovery of the association with the response to HCV therapy. The treatment of IFN-α, or IFN-λ1 inhibited HCV replication at similar levels at low concentrations (31). The combination treatment of IFN-α and *IL-29/28A* enhanced the antiviral effect against HCV replicon synergistically (32). In microarray analysis on ISG induction of IFN-α/β or IFN-λ1, IFN-λ1 showed a unique pattern of ISG expression compared to that of IFN-α/β (31). For example, a total of 19 genes, which were not detected in the IFN-α–treated cells, were specifically up-regulated by IFN-λ1 at the late phase of treatment, indicating the signal pathway downstream of IL-28R1 could differ from that of IFN-α and possess a important biological function, although the pattern of signal transduction currently thought to be similar to that of IFN-α R1/2 (33). Further studies are needed to elucidate the biological consequences of these differences.

As described above, HCV replication is inhibited by the antiviral effects of IFN-λ. IFN-λ might have potential as a therapeutic agent against chronic hepatitis C in patients. A pegylated IFN-λ1 has already been tried against chronic hepatitis C in phase 1B trials (34). Interestingly, sufficient antiviral effects were observed but not severe side effects. The expression pattern of the IFN-λ receptor is restricted in specific organs. The high expression of the receptor was observed in the pancreas, liver, prostate, or thyroid, whereas the central nerve system (the bone marrow or the brain) showed the low expression (27, 28). These results could explain the avoidance of severe toxicity induced by IFN-α/β.

11. Conclusions

The recent discovery revealed by GWAS technology provides the unexpected role of *IL-28B* in HCV infection. The findings could be strong evidence to enhance the development of a novel therapeutic strategy and basic studies on IFN-λs. The SNPs around the *IL-28B* gene could improve the diagnostics for the prediction of spontaneous clearance and the response to anti-HCV treatment. However, approximately 20% – 30% of the total homozygotes with the risk alleles in Caucasians and 20% of heterozygotes/homozygotes with risk alleles in the Japanese population achieved a SVR and vice versa (21 – 24), indicating that the response to a combination therapy is not inevitably restricted because of genetic factors. To improve the prediction rate, especially, host epigenetic, rare SNPs, mutations, or viral factors are eligible candidates to consider when trying to establish an adequate tailor-made therapy. Although the strongly associated SNPs may have a big impact on the type of therapy and outcome, this is the first step in the tailor-made therapy for HCV infection. Further functional studies of IFN-λs and the significant SNPs should be investigated to improve the positive predictive value using the point mutation analysis of the targeted polymorphisms (35). For applying a practical tailor-made therapy, it is also necessary to reveal the cause of exceptional cases that do not follow the *IL-28B* genotyping.

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