

Polymorphism Near the Interleukin-28B Gene and Anti-Hepatitis C Viral Response

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Abstract

In a recent genome-wide association study, single nucleotide polymorphisms (SNPs) located near the interleukin-28B gene (*IL28B*), which encodes type III interferon (IFN) $\lambda 3$, were shown to be strongly associated with a viral response to pegylated IFN α (PEG-IFN α) and ribavirin (RBV) combination therapy and spontaneous viral clearance in patients chronically and acutely infected with hepatitis C virus (HCV), respectively. The global distribution of allele frequencies shows a remarkable pattern, in which a favorable allele is nearly fixed in East Asia, has an intermediate frequency in Europe, and is least frequent in Africa. Although the underlying mechanisms responsible for viral responses associated with *IL28B* SNPs have not been completely elucidated, IFN-stimulated gene expression in patients with unfavorable *IL28B* genotypes tends to be high at baseline and is insufficiently induced by exogenous IFN administration, resulting in poor treatment outcomes. Clinically, triple therapy with PEG-IFN α /RBV together with direct-acting antiviral agents (DAAs) is currently used to treat chronic hepatitis C as a first-line therapy. Although the predictive power of *IL28B* status may be attenuated, the *IL28B* genotype will remain relevant to the outcomes of DAA therapy when used in combination with PEG-IFN α as a backbone. Even with the introduction of IFN-free therapies with a new class of highly effective DAAs, *IL28B* SNPs are still useful predictors of treatment outcomes and can be used to individualize treatment strategies to maximize cost-effectiveness and identify patients at risk of being refractory to treatment. This review summarizes the current understanding of the clinical significance and role of *IL28B* in HCV infection and response to therapy.

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Keywords: *IL28B*; IFN λ ; Innate immunity; Interferon-stimulated gene.

Abbreviations: HCV, hepatitis C virus; IFN, interferon; PEG-IFN α , pegylated interferon α ; RBV, ribavirin; DAA, direct-acting antiviral agent; NVR, nonviral response; GWAS, genome-wide association study; SNP, single nucleotide polymorphism; IL, interleukin; SVR, sustained viral response; Jak-STAT, Janus kinase-signal transducer and activator of transcription; ISG, interferon-stimulated gene; *RIG-I*, retinoic acid-inducible gene I; *MDA5*, melanoma differentiation-associated gene 5; TLR, toll-like receptor; IPS-1, interferon β promoter stimulator 1. Received: 13 February 2013; Revised: 15 May 2013; Accepted: 17 May 2013

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Introduction

Hepatitis C virus (HCV) infection is a global epidemic, with 130–170 million people currently affected worldwide. It is a common cause of chronic hepatitis, which can progress to liver cirrhosis and hepatocellular carcinoma in many patients.¹ During the last two decades, interferon (IFN)-based therapy has been used to treat chronic hepatitis C with the goal of altering the natural history of this disease. HCV eradication with IFN-based therapy has been shown to prevent hepatocellular carcinoma,^{2,3} and triple therapy with pegylated IFN α (PEG-IFN α) and ribavirin (RBV) together with direct-acting antiviral agents (DAAs) is currently used to treat chronic hepatitis C as a first-line therapy. Even with this newest standard of treatment that incorporates DAAs, a response to PEG-IFN α /RBV remains essential to obtain an adequate outcome; non-responders (defined as failure to clear HCV RNA from serum 24 weeks after initiating therapy) and particularly null responders (defined as failure to decrease HCV RNA by <2 logs at 24 weeks after initiating therapy) to PEG-IFN α /RBV also poorly respond to triple therapy that incorporates DAAs. Therefore, determining predictive factors and understanding the mechanisms responsible for responses to PEG-IFN α /RBV remain important for making treatment strategy decisions for individual patients.

In a recent genome-wide association study (GWAS), single nucleotide polymorphisms (SNPs) located near the interleukin-28B gene (*IL28B*), which encodes type III IFN $\lambda 3$, were shown to be strongly associated with a virological response to PEG-IFN α /RBV combination therapy.^{4–7} Patients infected with HCV genotype 1 who had favorable *IL28B* genotypes (rs12979860 CC or rs8099917 TT) had significantly better responses to PEG-IFN α /RBV combination therapy than those with other *IL28B* variants.^{4–6} In addition, spontaneous clearance of acute HCV infection was more likely in patients with favorable *IL28B* genotypes.⁸

In contrast, the rs12979860 CT and TT or rs8099917 TG and GG genotypes (unfavorable *IL28B* genotypes) were strongly associated with a non-viral response (NVR) (defined as patients with detectable HCV RNA during and after treatment) to PEG-IFN α /RBV.⁶ However, the mechanisms involved in resistance to PEG-IFN α /RBV associated with unfavorable *IL28B* genotypes have not been completely elucidated.

Clinical significance of *IL28B* SNPs

Marked differences in HCV clearance and response to treatment among ethnic groups have long suggested a role for host genetic factors. A recent GWAS approach used high-throughput

genotyping ranging from 300,000 to 1,000,000 SNPs for each sample. This approach could detect factors strongly associated with disease susceptibility and drug responses without any *a priori* hypotheses with regard to causative SNPs. Four independent GWASs also assessed genetic variations with regard to the responses to PEG-IFN α /RBV combination therapy for patients with chronic hepatitis C (Table 1).⁴⁻⁷ In all these studies, the conclusive findings were that SNPs in or near the *IL28B* gene were strong pretreatment predictors of a sustained virological response (SVR) and NVR. These studies identified two SNPs near the *IL28B* gene (rs12979860 C/T and rs8099917 T/G). Patients homozygous for the major allele with a so-called favorable genotype (rs12979860 CC or rs8099917 TT) were twice as likely to achieve an SVR compared with patients with a minor risk allele (rs12979860 TT or CT or rs8099917 GG or TG) after PEG-IFN α /RBV combination therapy. The *IL28B* genotype was also strongly associated with spontaneous clearance of HCV infection. In acute HCV infection, patients who were homozygous for the major allele were more likely to spontaneously clear the virus than those with the minor allele.⁸ Among patients with a minor risk allele, the differences in SVR and spontaneous clearance rates were less notable (less than twice) between heterozygotes (rs12979860 CT or rs8099917 TG) and homozygotes (rs12979860 TT or rs8099917 GG).

With regard to the *IL28B* genotype, the differences in treatment responses to PEG-IFN α /RBV therapy are remarkable in patients with HCV genotype 1. The *IL28B* SNP is also associated with an SVR in patients infected with the non-1 HCV genotype.⁹⁻¹³ However, this association is less notable, particularly in patients with genotype 2/3 with rapid virological responses (defined as HCV RNA negative at treatment week 4 by a sensitive PCR-based quantitative assay)⁹ and patients with genotype 2a.¹⁰

The allele frequency varies markedly across ethnic groups. Accordingly, differences among population groups were noted in SVR rates in patients treated with PEG-IFN α /RBV and spontaneous HCV clearance. The global distribution of allele frequencies shows a remarkable pattern, in which a favorable allele (C in rs12979860 and T in rs8099917) that results in a greater therapeutic response and natural HCV clearances nearly fixed throughout East Asia, has an intermediate frequency in Europe, and is least frequent in Africa.⁸ HCV clearance occurs in 36.4% of individuals of non-African ancestry infected with HCV but in only 9.3% of individuals of African ancestry infected with HCV, which may be explained, in part, by the observed differences in allelic frequency in these ethnic groups.

Both SNPs at rs12979860 and rs8099917 are in strong linkage disequilibrium, but the rs8099917 allele frequency

differs among population groups worldwide; thus, its predictive power may vary between diverse cohorts.¹⁴ Because of the significant effect of rs12979860 on treatment outcome, determining this SNP may be sufficient for predicting an SVR in patients infected with HCV genotype 1 who are treated with PEG-IFN α /RBV combination therapy.^{15,16} However, haplotypes that include both SNPs may be more accurate than either SNP alone. Although the predictive value for the treatment outcome could not be improved in patients with the rs12979860 CC genotype (homozygous carrier for the responder allele) by also determining the rs8099917 SNP, there was evidence that a significant proportion of heterozygous carriers of the rs12979860 T non-responder allele could profit with respect to SVR prediction by also determining the rs8099917 SNP.^{15,16}

Pretreatment factors associated with *IL28B* genotypes

An unfavorable *IL28B* genotype is associated with several host and viral factors, most of which are unfavorable factors for the response to PEG-IFN α /RBV therapy. However, the mechanisms responsible for the coexistence of these unfavorable factors are currently unknown. Patients with unfavorable *IL28B* genotypes have significantly higher γ -glutamyltranspeptidase levels,¹⁷ lower low-density lipoprotein cholesterol levels,^{18,19} a higher frequency of hepatic steatosis,²⁰ glutamine or histidine mutations at amino acid position 70 in the HCV core region,²¹ and one or no mutations in the IFN sensitivity-determining region in the HCV non-structural 5A gene.²¹

Liver fibrosis and *IL28B* genotypes

An association between the *IL28B* genotype and risk of liver fibrosis progression remains controversial. A recent cross-sectional study analyzed 1329 patients with HCV genotype 1 and found no relationship between *IL28B* SNPs and advanced fibrosis.²² Similarly, Marabita *et al.* estimated the fibrosis progression rate in 247 patients with known dates of infection and showed that the *IL28B* genotype had no effect on the risk of developing advanced fibrosis.²³ A recent cohort study revealed a significant relationship between an unfavorable *IL28B* genotype and a slow fibrosis progression rate; however, this relationship was found only in patients infected with a genotype other than genotype 1, and not in genotype 1-infected patients.²⁴ Taken together, SNPs near the *IL28B* gene do not appear to be closely associated with liver fibrogenesis in HCV genotype 1-monoinfected patients.²⁵

Table 1. Four GWASs identifying SNPs associated with PEG-IFN/RBV therapy

| | Ge et al. ⁴ | Suppiah et al. ⁵ | Tanaka et al. ⁶ | Rauch et al. ⁷ |
|--------------------|----------------------------|-----------------------------|----------------------------|---------------------------|
| Year | 2009 | 2009 | 2009 | 2010 |
| Ancestry | Caucasian/African/Hispanic | Caucasian | Japanese | Caucasian |
| Number of patients | 1137 | 293 | 142 | 465 |
| SNP | rs12979860 | rs8099917 | rs8099917 | rs8099917 |
| Odds ratio | 3.1 | 1.98 | 12.1 | 5.20 |
| <i>p</i> value | 1.21×10^{-28} | 7.06×10^{-8} | 3.11×10^{-15} | 5.47×10^{-8} |
| HCV genotype | 1 | 1 | 1 | 1, 2, 3, 4 |

IL28B (IFNλ3)

The *IL28B* gene, also known as the *IFNλ3* gene, is a member of a highly homologous type III IFNλ family consisting of three members: *IL29 (IFNλ1)*, *IL28A (IFNλ2)*, and *IL28B (IFNλ3)*.²⁶ All of these are located on chromosome 19.^{27,28} The amino acid sequences of *IL28A* and *IL28B* have 96% homology, and both are 81% identical to the amino acid sequence of *IL29*.²⁸ Similar to IFNα, IFNλs are triggered by viral infections and induce antiviral activity through both the innate and adaptive immune systems.^{29,30}

Compared with type I IFN, IFNλs are produced by relatively restricted cell types, including hepatocytes, intestinal epithelial cells, and dendritic cells [including BDCA3(+) cells].³¹ In addition, the type III IFN receptor, a heterodimer of the *IL10* and *IL28* receptors, is expressed in relatively restricted cell types, including hepatocytes, intestinal epithelial cells, and plasmacytoid dendritic cells.³² Both type I IFN and IFNλs activate the Janus kinase–signal transducer and activator of transcription (Jak–STAT) pathway^{27,33} after binding to type I and type III receptors, respectively, and induce a significant number of IFN-stimulated genes (ISGs).³⁴ Although both types of IFN induce an overlapping set of ISGs, substantial differences have been observed between type I IFN and IFNλs in terms of the gene expression induced by them.³⁵

The kinetics of ISG induction also differs between IFNα and IFNλs. The initial change in ISG expression in response to IFNλs is weaker; however, it gradually increases and is subsequently maintained.³⁴ In contrast, the kinetics of ISGs in response to IFNα shows a steep peak after induction, as reflected by an earlier stronger induction, which is then followed by a rapid decline.³⁶ Because of these differences between type I and III IFNs, IFNλs may play roles in antiviral activity that are distinct from type I IFNs.

The relationship between *IL28B* genotypes and basal levels of *IL28* expression remains controversial. However, *IL28B* promoter activity is reportedly lower with unfavorable *IL28B* SNPs,^{37,38} and *ex vivo* induction of *IL28B* transcripts by poly I:C stimulation is lower in peripheral blood mononuclear cells derived from patients with chronic hepatitis C with unfavorable *IL28B* genotypes.³⁸ Moreover, BDCA3(+) dendritic cells produce larger amounts of IFNλs upon HCV stimulation in patients with favorable *IL28B* genotypes,³¹ suggesting that the activity of IFNλ induction may affect treatment outcomes associated with *IL28B* genotypes.

IFNλ induces intracellular responses similar to those of IFNα but in fewer cell types because of differences in the receptor distribution pattern. This could potentially result in an improved safety profile. In phase I proof-of-concept studies of PEG-IFNλ1 with or without RBV in patients with chronic HCV genotype 1 infections, PEG-IFNλ1 showed an improved safety profile with minimal flu-like symptoms and no significant hematologic changes other than RBV-associated decreases in hemoglobin compared with PEG-IFNα.³⁹

Innate immunity and IL28B genotypes: proposed role for IL28B in response to IFN

The innate immune system plays an essential role in host antiviral defense against HCV infection.⁴⁰ The retinoic acid-inducible gene I (*RIG-I*), a cytoplasmic RNA helicase, related melanoma differentiation-associated gene 5 (*MDA5*), and toll-like receptor 3 (TLR3) play essential roles in initiating the

host antiviral response by detecting intracellular viral RNA^{41,42} (Fig. 1). The IFNβ promoter stimulator 1 (IPS-1), also called the caspase-recruiting domain adaptor inducing IFNβ, mitochondrial antiviral signaling protein, or virus-induced signaling adaptor, is an adaptor molecule. IPS-1 connects RIG-I sensing to downstream signaling, resulting in IFNβ and IFNλ gene activation.^{43–46}

IFNβ binds to the type I IFN receptor, whereas IFNλ binds to the type III IFN receptor comprising an *IL10R–IL28R* receptor complex.²⁷ Both the receptors activate the Jak–STAT pathway, which upregulates a large number of ISGs by activating the IFN-stimulated response element.⁴⁷ In recent reports, IFNλ and not type I IFN was shown to be primarily induced by HCV infection, which subsequently enhanced ISG expression in primary human hepatocytes.⁴⁸ The degree of hepatic IFNλ induction was closely correlated with the strength of the ISG response.⁴⁹ Thus, hepatic IFNλs may be essential for inducing ISGs and subsequent HCV eradication.

Considerable attention has been paid to the relationships between ISG induction, *IL28B* genotypes, and treatment outcomes. Several reports, including ours, have demonstrated that intrahepatic gene expression levels of ISGs and levels of cytoplasmic viral sensors such as RIG-I and MDA5 (known as RIG-I like receptors) were markedly upregulated at baseline and were poorly induced by exogenous IFNα administration in NVR when treated with PEG-IFNα/RBV combination therapy.^{50,51} Similarly, hepatic expression of ISGs and RIG-I-like receptors in patients with unfavorable *IL28B* genotypes was significantly upregulated compared with that in patients with favorable *IL28B* genotypes.^{52–54}

However, even in a subgroup with unfavorable *IL28B* genotypes, ISG and RIG-I expression was significantly higher in NVRs than in virological responders.⁵² Similar tendencies were observed in a subgroup with favorable *IL28B* genotypes, in which ISG and RIG-I expression was higher in NVRs than in virological responders.⁵² Therefore, ISG and RIG-I expression is likely to be the best predictor of treatment response regardless of the *IL28B* genotype, and higher expression of these genes may be a more fundamental phenomenon for IFN resistance.^{51,52}

ISG upregulation by endogenous IFN in the presence of intracellular HCV and the poor response of ISGs to exogenous IFN are closely associated with essential mechanisms. An unfavorable *IL28B* SNP results in the continuous activation of a subset of ISGs in the presence of intracellular HCV.⁵² However, this level of expression is insufficient to eliminate HCV from infected cells because it may upregulate IFN-inhibitory molecules such as the suppressor of cytokine signaling 3 (SOCS3) and the protein inhibitor of activated STAT (PIAS), thereby reducing sensitivity to IFN signaling.⁵⁵ Therefore, infected cells are not only unable to clear the virus but are also unable to promote stronger ISG induction when IFN is exogenously administered during therapy.⁵⁰

Recently, *IFNλ4*, a new IFN gene, was discovered by RNA sequencing using primary human hepatocytes that were activated with synthetic double-stranded RNA to mimic HCV infection.⁵⁶ The IFNλ4 protein has 179 amino acids and is a frameshift variant that is created by ss469415590 (ΔG); a dinucleotide variant, ss469415590 (TT or ΔG), is in high linkage disequilibrium with rs12979860. IFNλ4 induces STAT1 and STAT2 phosphorylation and upregulates ISGs by activating the IFN-stimulated response element. However, this preactivation of IFN signaling induced by IFNλ4 impairs HCV clearance and prevents further activation by exogenous

type I and type III IFNs, which is required for efficient HCV clearance (Fig. 1). Although the mechanisms responsible for this impairment are unclear, the innate immune response in patients with unfavorable *IL28B* SNPs may have adapted to a different equilibrium state compared with that in patients with favorable *IL28B* SNPs.

***IL28B* SNPs and triple therapy with DAAs**

The *IL28B* genotype status is one of the most important pretreatment predictors of response to PEG-IFN α /RBV therapy. The treatment regimen for chronic HCV has changed dramatically with the development of DAAs, which directly inhibit specific HCV or host molecules required during the steps of the HCV lifecycle, including HCV NS3-4A protease, NS5B polymerase, and NS5A phosphoprotein, as well as host cell proteins involved in HCV replication.⁵⁷ Because the predictive role of *IL28B* SNPs is based on PEG-IFN α /RBV combination therapy, the key question is whether the *IL28B* genotype will remain a useful predictor of treatment outcomes for triple therapy with DAAs.

A recent report revealed that the SVR rate with triple therapy with telaprevir/PEG-IFN α /RBV was high (84%) for patients with a favorable *IL28B* genotype, irrespective of any substitution in HCV core aa70. In patients with unfavorable *IL28B* genotypes, those with HCV core aa70 wild-type achieved a high SVR rate (50%), whereas those with HCV core aa70 mutant achieved an SVR rate of only 12%. This suggests that genetic variations near the *IL28B* gene and an HCV core amino acid substitution are predictors of SVR to triple therapy for patients infected with HCV genotype 1b.⁵⁸

However, for patients enrolled in the PILLAR study, a phase IIb study of simeprevir (TMC435; a second-generation NS3-4A protease inhibitor) in combination with PEG-IFN α /RBV for treatment-naïve HCV genotype 1 patients, high rates of viral response were achieved for all patients regardless of the *IL28B* genotype.

Therefore, the predictive power of *IL28B* status may be attenuated in the setting of triple therapy with more potent DAAs.^{58,59} However, the *IL28B* genotype will remain relevant to the outcomes of DAA therapy when used in combination with PEG-IFN α as a backbone. Because an unfavorable *IL28B* SNP is refractory to a response to IFN signaling and results in failure to suppress the emergence of resistant HCV mutants, *IL28B* genotyping could be helpful in identifying patients who are poor candidates for triple therapy.

***IL28B* SNP and IFN-free DAA combination therapy**

With the development of more potent DAA combinations, IFN-free therapy is likely to be approved in the near future. This raises the question of whether *IL28B* genotyping will remain a useful predictor of treatment responses. Interim results from a large study designed to evaluate the combination of BI-201335 (NS3-4A protease inhibitor) and BI-207127 (non-nucleoside polymerase inhibitor) with or without RBV in chronic hepatitis C patients demonstrated a clear difference in SVR between favorable and unfavorable *IL28B* genotypes in HCV-1a patients.⁶⁰ In that study, *IL28B*, HCV genotype, sex, and pretreatment γ -glutamyltranspeptidase levels were identified as factors independently associated with SVR by multivariate analysis. Because the low SVR rates

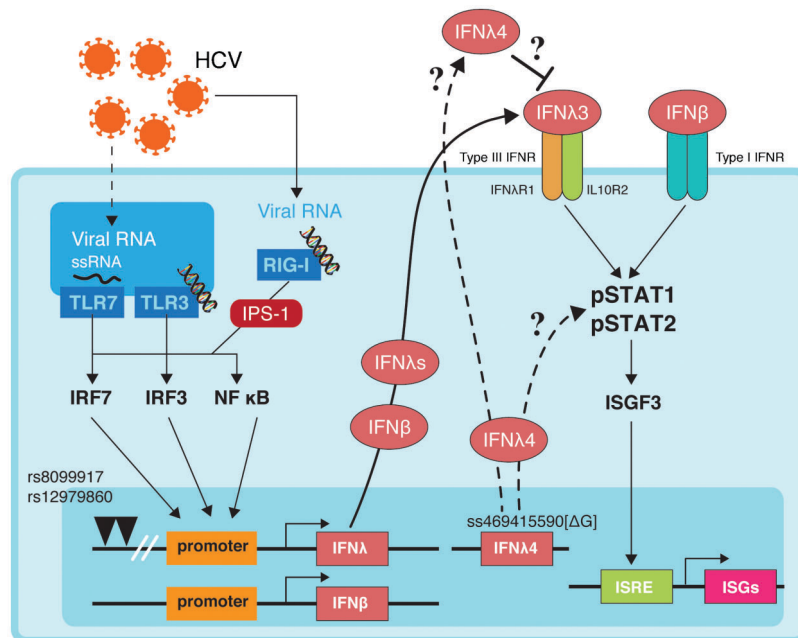


Fig. 1. Host antiviral innate defense system proposed from *in vitro* experiments. Upon viral infection, cytoplasmic viral sensors (RIG-I) and toll-like receptors (TLRs) detect viral pathogens, which results in IFN β and IFN λ s gene activation via the adaptor molecule IPS-1. IFN β binds to type I IFN receptors, whereas IFN λ s bind to type III IFN receptors comprising IL10R–IL28R receptor complexes. Both receptors activate the Jak–STAT pathway, which upregulates a large number of ISGs by activating the IFN-stimulated response element (ISRE). *IL28B* promoter activity is reportedly lower with unfavorable *IL28B* SNPs. RIG-I and ISG expressions in patients with unfavorable *IL28B* genotypes tend to be high at baseline and are insufficiently induced by exogenous IFN administration, resulting in poor treatment outcomes with IFN-based therapy. IFN λ 4 is created by ss469415590 variant (Δ G), which is in high linkage disequilibrium with rs12979860. Although IFN λ 4 induces ISGs by activating ISRE, this preactivation of IFN signaling impairs HCV clearance and prevents further activation by exogenous type I and type III IFNs.

observed for HCV-1a patients with unfavorable *IL28B* genotypes resulted from virological breakthrough in most patients, the *IL28B* status influenced the emergence of resistant mutant HCVs with IFN-free therapy.

However, this effect of the *IL28B* genotype was not observed in patients infected with HCV-1b, which is more susceptible to DAAs than HCV-1a. Moreover, there is also increasing evidence that combination therapy with more potent DAA regimens may reduce the importance of the *IL28B* genotype as a predictor of treatment response. A recent study of the combination of sofosbuvir (nucleoside polymerase inhibitor) with an NS5A inhibitor (daclatasvir or GS-5885) reported an SVR12 (defined as HCV RNA negative 12 weeks after cessation of treatment) of 100% for HCV-1 treatment-naïve patients and prior null responders with unfavorable *IL28B* genotypes.^{61,62} Another recent study of the combination of ABT-450/r (NS3-4A protease inhibitor/ritonavir), ABT-267 (NS5A inhibitor), ABT-333 (polymerase inhibitor), and RBV reported an SVR12 of 95% for patients with HCV-1 who were null responders to prior treatment.⁶³

Although the association between *IL28B* genotypes and treatment outcomes has been attenuated by the development of IFN-free therapy with more potent DAAs, *IL28B* genotyping may continue to be useful for identifying patients who should be treated with these potent but expensive regimens as first-line therapy or those who can be treated with shorter or less-expensive regimens.

Conclusions

SNPs upstream of the *IL28B* gene are associated with spontaneous HCV clearance and response to PEG-IFN α /RBV combination therapy. Although the underlying mechanisms responsible for NVRs associated with unfavorable *IL28B* SNPs have not been completely elucidated, ISG expression in patients with unfavorable *IL28B* genotypes tends to be high at baseline and is insufficiently induced by exogenous IFN administration, resulting in poor treatment outcomes with IFN-based therapy. Even with the introduction of the new class of highly effective DAAs, *IL28B* SNPs remain useful predictors of treatment outcomes and can be used to individualize treatment strategies to maximize cost-effectiveness and identify patients at risk of being refractory to treatment due to the emergence of multidrug-resistant HCV.

Conflict of interest

Dr Asahina and Dr Kakinuma belong to a donation-funded department funded by Chugai Pharmaceutical Co., Ltd., Toray Industries, Inc., Bristol-Myers Squibb Company, Dainippon Sumitomo Pharma Co., Ltd., and MSD K.K.

Author contributions

Manuscript writing (YA), critical discussion (MN, SK, MW).

References

- [1] Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, *et al.* Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990;12:671–675.

- [2] Asahina Y, Tsuchiya K, Tamaki N, Hirayama I, Tanaka T, Sato M, *et al.* Effect of aging on risk for hepatocellular carcinoma in chronic hepatitis C virus infection. *Hepatology* 2010;52:518–527.
- [3] Asahina Y, Tsuchiya K, Nishimura T, Muraoka M, Suzuki Y, Tamaki N, *et al.* α -fetoprotein levels after interferon therapy and risk of hepatocarcinogenesis in chronic hepatitis C. *Hepatology* 2013 Apr 8.
- [4] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, *et al.* Genetic variation in *IL28B* predicts hepatitis C treatment-induced viral clearance. *Nature* 2009;461:399–401.
- [5] Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, *et al.* *IL28B* is associated with response to chronic hepatitis C IFN-alpha and ribavirin therapy. *Nat Genet* 2009;41:1100–1104.
- [6] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, *et al.* Genome-wide association of *IL28B* with response to pegylated IFN-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009;41:1105–1109.
- [7] Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, *et al.* Genetic variation in *IL28B* is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology* 2010;138:1338–1345.
- [8] Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O’Huigin C, *et al.* Genetic variation in *IL28B* and spontaneous clearance of hepatitis C virus. *Nature* 2009;461:798–801.
- [9] Mangia A, Thompson AJ, Santoro R, Piazzolla V, Tillmann HL, Patel K, *et al.* An *IL28B* polymorphism determines treatment response of hepatitis C virus genotype 2 or 3 patients who do not achieve a rapid virologic response. *Gastroenterology* 2010;139:821–827.
- [10] Sakamoto N, Nakagawa M, Tanaka Y, Sekine-Osajima Y, Ueyama M, Kurosaki M, *et al.* Association of *IL28B* variants with response to pegylated-interferon alpha plus ribavirin combination therapy reveals intersubgenotypic differences between genotypes 2a and 2b. *J Med Virol* 2011;83:871–878.
- [11] Kawaoka T, Hayes CN, Ohishi W, Ochi H, Maekawa T, Abe H, *et al.* Predictive value of the *IL28B* polymorphism on the effect of interferon therapy in chronic hepatitis C patients with genotypes 2a and 2b. *J Hepatol* 2011;54:408–414.
- [12] Sarrazin C, Susser S, Doehring A, Lange CM, Müller T, Schlecker C, *et al.* Importance of *IL28B* gene polymorphisms in hepatitis C virus genotype 2 and 3 infected patients. *J Hepatol* 2011;54:415–421.
- [13] Asselah T, De Muynck S, Broët P, Masliah-Planchon J, Blanluet M, Bièche I, *et al.* *IL28B* polymorphism is associated with treatment response in patients with genotype 4 chronic hepatitis C. *J Hepatol* 2012;56:527–532.
- [14] Melis R, Fauron C, McMillin G, Lyon E, Shirts B, Hubley LM, *et al.* Simultaneous genotyping of rs12979860 and rs8099917 variants near the *IL28B* locus associated with HCV clearance and treatment response. *J Mol Diagn* 2011;13:446–451.
- [15] Fischer J, Böhm S, Scholz M, Müller T, Witt H, George J, *et al.* Combined effects of different interleukin-28B gene variants on the outcome of dual combination therapy in chronic hepatitis C virus type 1 infection. *Hepatology* 2012;55:1700–1710.
- [16] Ladero JM, Martin EG, Fernández C, Carballo M, Devesa MJ, Martínez C, *et al.* Predicting response to therapy in chronic hepatitis C: an approach combining interleukin-28B gene polymorphisms and clinical data. *J Gastroenterol Hepatol* 2012;27:279–285.
- [17] Abe H, Ochi H, Maekawa T, Hayes CN, Tsuge M, Miki D, *et al.* Common variation of *IL28* affects gamma-GTP levels and inflammation of the liver in chronically infected hepatitis C virus patients. *J Hepatol* 2010;53:439–443.
- [18] Li JH, Lao XQ, Tillmann HL, Rowell J, Patel K, Thompson A, *et al.* Interferon-lambda genotype and low serum low-density lipoprotein cholesterol levels in patients with chronic hepatitis C infection. *Hepatology* 2010;51:1904–1911.
- [19] Aizawa Y, Yohizawa K, Aida Y, Ishiguro H, Abe H, Tsubota A. Genotype rs8099917 near the *IL28B* gene and amino acid substitution at position 70 in the core region of the hepatitis C virus are determinants of serum apolipoprotein B-100 concentration in chronic hepatitis C. *Mol Cell Biochem* 2012;360:9–14.
- [20] Tillmann HL, Patel K, Muir AJ, Guy CD, Li JH, Lao XQ, *et al.* Beneficial *IL28B* genotype associated with lower frequency of hepatic steatosis in patients with chronic hepatitis C. *J Hepatol* 2011;55:1195–1200.
- [21] Maekawa S, Sakamoto M, Miura M, Kadokura M, Sueki R, Komase K, *et al.* Comprehensive analysis for viral elements and interleukin-28B polymorphisms in response to pegylated interferon plus ribavirin therapy in hepatitis C virus 1B infection. *Hepatology* 2012;56:1611–1621.
- [22] Thompson AJ, Clark PJ, Fellay J, Muir AJ, Tillmann HL, Patel K, *et al.* *IL28B* genotype is not associated with advanced hepatic fibrosis in chronic hepatitis C patients enrolled in the IDEAL study. *Hepatology* 2010;52:437A–438A.
- [23] Marabita F, Aghemo A, Nicola SD, Rumi MG, Cheroni C, Scavelli R, *et al.* Genetic variation in the interleukin-28B gene is not associated with fibrosis progression in patients with chronic hepatitis C and known date of infection. *Hepatology* 2011;54:1127–1134.
- [24] Bochud PY, Bibert S, Kutalik Z, Patin E, Guernon J, Nalpas B, *et al.* *IL28B* alleles associated with poor HCV clearance protect against inflammation and

- fibrosis in patients infected with non-1 HCV genotypes. *Hepatology* 2012;55:384–394.
- [25] Asahina Y, Tsuchiya K, Nishimura T, Muraoka M, Suzuki Y, Tamaki N, et al. Genetic variation near interleukin 28B and the risk of hepatocellular carcinoma in patients with chronic hepatitis C. *J Gastroenterol* 2013 in press.
- [26] Gad HH, Dellgren C, Hamming OJ, Vends S, Paludan SR, Hartmann R. Interferon- λ is functionally an interferon but structurally related to the interleukin-10 family. *J Biol Chem* 2009;284:20869–20875.
- [27] Kottenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, et al. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat Immunol* 2003;4:69–77.
- [28] Sheppard P, Kindsvogel W, Xu W, Henderson K, Schlutsmeyer S, Whitmore TE, et al. IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nat Immunol* 2003;4:63–68.
- [29] Yoshimoto K, Kishida T, Nakano H, Matsui M, Shin-Ya M, Shimada T, et al. Interleukin-28B acts synergistically with cisplatin to suppress the growth of head and neck squamous cell carcinoma. *J Immunother* 2011;34:139–148.
- [30] Morrow MP, Yan J, Pankhong P, Shedlock DJ, Lewis MG, Talbott K, et al. IL-28B/IFN-lambda 3 drives granzyme B loading and significantly increases CTL killing activity in macaques. *Mol Ther* 2010;18:1714–1723.
- [31] Yoshio S, Kanto T, Kuroda S, Matsubara T, Higashitani K, Kakita N, et al. Human BDCA3(+) dendritic cells are a potent producer of IFN- λ in response to hepatitis C virus. *Hepatology* 2013;57:1705–1715.
- [32] Sommereyns C, Paul S, Staeheli P, Michiels T. IFN-lambda (IFN- λ) is expressed in a tissue-dependent fashion and primarily acts on epithelial cells in vivo. *PLoS Pathog* 2008;4:e1000017.
- [33] Zhang L, Jilg N, Shao RX, Lin W, Fusco DN, Zhao H, et al. IL28B inhibits hepatitis C virus replication through the JAK-STAT pathway. *J Hepatol* 2011;55:289–298.
- [34] Zhou Z, Hamming OJ, Ank N, Paludan SR, Nielsen AL, Hartmann R. Type III interferon (IFN) induces a type I IFN-like response in a restricted subset of cells through signaling pathways involving both the Jak-STAT pathway and the mitogen-activated protein kinases. *J Virol* 2007;81:7749–7758.
- [35] Thomson SJ, Goh FG, Banks H, Krausgruber T, Kottenko SV, Foxwell BM, et al. The role of transposable elements in the regulation of IFN- λ 1 gene expression. *Proc Natl Acad Sci USA* 2009;106:11564–11569.
- [36] Marcello T, Grakoui A, Barba-Spaeth G, Machlin ES, Kottenko SV, MacDonald MR, et al. Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. *Gastroenterology* 2006;131:1887–1898.
- [37] Sugiyama M, Tanaka Y, Wakita T, Nakanishi M, Mizokami M. Genetic variation of the IL-28B promoter affecting gene expression. *PLoS One* 2011;6:e26620.
- [38] Asahina Y, Murakawa M, Sakamoto N, Kakinuma S, Nakagawa M, Azuma S, et al. Impaired IL28B gene induction and poor IL28B promoter activity influenced by the IL28B minor allele are closely associated with a null response to interferon in chronic hepatitis C patients. *Hepatology* 2012;56:697A.
- [39] Muir AJ, Shiffman ML, Zaman A, Yoffe B, de la Torre A, Flamm S, et al. Phase 1b study of pegylated interferon lambda 1 with or without ribavirin in patients with chronic genotype 1 hepatitis C virus infection. *Hepatology* 2010;52:822–832.
- [40] Biron CA. Initial and innate responses to viral infections – pattern setting in immunity or disease. *Curr Opin Microbiol* 1999;2:374–381.
- [41] Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol* 2004;5:730–737.
- [42] Yoneyama M, Kikuchi M, Matsumoto K, Imaizumi T, Miyagishi M, Taira K, et al. Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. *J Immunol* 2005;175:2851–2858.
- [43] Meylan E, Curran J, Hofmann K, Moradpour D, Binder M, Bartschlagler R, et al. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. *Nature* 2005;437:1167–1172.
- [44] Kawai T, Takahashi K, Sato S, Coban C, Kumar H, Kato H, et al. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nat Immunol* 2005;6:981–988.
- [45] Seth RB, Sun L, Ea CK, Chen ZJ. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. *Cell* 2005;122:669–682.
- [46] Xu LG, Wang YY, Han KJ, Li LY, Zhai Z, Shu HB. VISA is an adapter protein required for virus-triggered IFN-beta signaling. *Mol Cell* 2005;19:727–740.
- [47] Rehermann B. Hepatitis C virus versus innate and adaptive immune responses: a tale of coevolution and coexistence. *J Clin Invest* 2009;119:1745–1754.
- [48] Park H, Serti E, Eke O, Muchmore B, Prokunina-Olsson L, Capone S, et al. IL29 is the dominant type III interferon produced by hepatocytes during acute hepatitis C virus infection. *Hepatology* 2012;56:2060–2070.
- [49] Thomas E, Gonzalez VD, Li Q, Modi AA, Chen W, Nouredin M, et al. HCV infection induces a unique hepatic innate immune response associated with robust production of type III interferons. *Gastroenterology* 2012;142:978–988.
- [50] Asahina Y, Izumi N, Hirayama I, Tanaka T, Sato M, Yasui Y, et al. Potential relevance of cytoplasmic viral sensors and related regulators involving innate immunity in antiviral response. *Gastroenterology* 2008;134:1396–1405.
- [51] Dill MT, Duong FH, Vogt JE, Bibert S, Bochud PY, Terracciano L, et al. Interferon-induced gene expression is a stronger predictor of treatment response than IL28B genotype in patients with hepatitis C. *Gastroenterology* 2011;140:1021–1031.
- [52] Asahina Y, Tsuchiya K, Muraoka M, Tanaka K, Suzuki Y, Tamaki N, et al. Association of gene expression involving innate immunity and genetic variation in interleukin 28B with antiviral response. *Hepatology* 2012;55:20–29.
- [53] Honda M, Sakai A, Yamashita T, Nakamoto Y, Mizukoshi E, Sakai Y, et al. Hepatic ISG expression is associated with genetic variation in interleukin 28B and the outcome of IFN therapy for chronic hepatitis C. *Gastroenterology* 2010;139:499–509.
- [54] Abe H, Hayes CN, Ochi H, Maekawa T, Tsuge M, Miki D, et al. IL28 variation affects expression of interferon stimulated genes and peg-interferon and ribavirin therapy. *J Hepatol* 2011;54:1094–1101.
- [55] Sarasin-Filipowicz M, Oakeley EJ, Duong FH, Christen V, Terracciano L, Filipowicz W, et al. Interferon signaling and treatment outcome in chronic hepatitis C. *Proc Natl Acad Sci USA* 2008;105:7034–7039.
- [56] Prokunina-Olsson L, Muchmore B, Tang W, Pfeiffer RM, Park H, Dickensheets H, et al. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nat Genet* 2013;45:164–171.
- [57] Fowell AJ, Nash KL. Telaprevir: a new hope in the treatment of chronic hepatitis C? *Adv Ther* 2010;27:512–522.
- [58] Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, et al. Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. *Hepatology* 2010;52:421–429.
- [59] Chayama K, Hayes CN, Abe H, Miki D, Ochi H, Karino Y, et al. IL28B but not ITPA polymorphism is predictive of response to pegylated interferon, ribavirin, and telaprevir triple therapy in patients with genotype 1 hepatitis C. *J Infect Dis* 2011;204:84–93.
- [60] Zeuzem S, Asselah T, Angus P, Zarski JP, Larrey D, Müllhaupt B, et al. Faldaprevir (BI 201335), BI 207127 and ribavirin oral therapy for treatment-naïve HCV genotype 1: SOUND-C1 final results. *Antivir Ther* 2013. doi: 10.3851/IMP2567.
- [61] Sulkowski MS, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, et al. Sustained virologic response with daclatasvir plus sofosbuvir \pm ribavirin (RBV) in chronic HCV genotype (GT) 1-infected patients who previously failed telaprevir (TVR) or boceprevir (BOC). *J Hepatol* 2013;58:S570.
- [62] Gane EJ, Stedman CA, Hyland RH, Ding X, Pang PS, Symonds WT, et al. All-oral sofosbuvir-based 12-week regimens for the treatment of chronic HCV infection: the ELECTRON study. *J Hepatol* 2013;58:S6-S7.
- [63] Kowdley KV, Lawitz E, Poordad F, Cohen DE, Nelson D, Zeuzem S, et al. Safety and efficacy of interferon-free regimens of ABT-450/r, ABT-267, ABT-333 \pm ribavirin in patients with chronic HCV GT1 infection: results from the AVIATOR study. *J Hepatol* 2013;58:S2.