Review Article

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

Yasuhiro Asahina[1,2], Mina Nakagawa[1], Sei Kakinuma[1,2] and Mamoru Watanabe[1]

[1]*Department of Gastroenterology and Hepatology;* [2]*Department of Liver Disease Control, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo, Japan*

## 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) λ3, were shown to be strongly associated with a viral response to pegylated IFNα (PEG-IFNα) 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-IFNα/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-IFNα 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.



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

**Abbreviations:** HCV, hepatitis C virus; IFN, interferon; PEG-IFNα, pegylated interferonα; 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.

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**Correspondence to:** Yasuhiro Asahina, Department of Liver Disease Control, Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan. Tel: +81-3-5803-5877, Fax: +81-3-5803-0268, E-mail: asahina.gast@tmd.ac.jp

## 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 IFNα (PEG-IFNα) 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-IFNα/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-IFNα/RBV also poorly respond to triple therapy that incorporates DAAs. Therefore, determining predictive factors and understanding the mechanisms responsible for responses to PEG-IFNα/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 IFNλ3, were shown to be strongly associated with a virological response to PEG-IFNα/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-IFNα/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-IFNα/RBV.[6] However, the mechanisms involved in resistance to PEG-IFNα/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-IFNα/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-IFNα/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 (rs19979860 CT or rs8099917 TG) and homozygotes (rs19979860 TT or rs8099917 GG).

With regard to the *IL28B* genotype, the differences in treatment responses to PEG-IFNα/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-IFNα/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 clearanceis 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-IFNα/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-IFNα/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 nonstructural 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 |
| *p* value | $1.21 \times 10^{-28}$ | $7.06 \times 10^{-8}$ | $3.11 \times 10^{-15}$ | $5.47 \times 10^{-8}$ |
| 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-IFNα/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-IFNα/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-IFNα/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-IFNα/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-IFNα 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

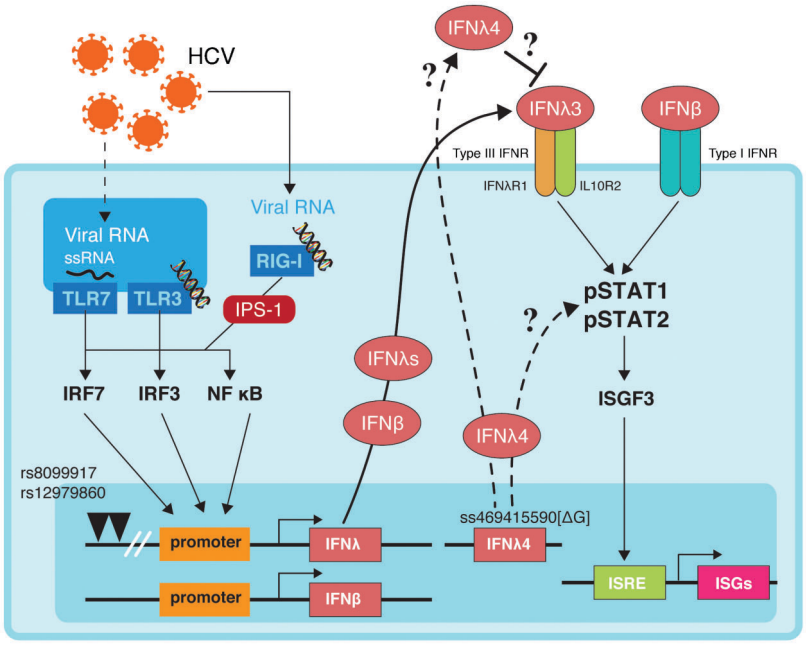


**Fig. 1. Host antiviral innate defense system proposed from *in vitro* experiments.** Upon viral infection, cytoplasmic viral sensors (RIG-I) and toll-like receptors (TLRs) detect viral pathogens, which results in IFNβ and IFNλs gene activation via the adaptor molecule IPS-1. IFNβ binds to type I IFN receptors, whereas IFNλs 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. IFNλ4 is created by ss469415590 variant (ΔG), which is in high linkage disequilibrium with rs12979860. Although IFNλ4 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 *IL28*B 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$\alpha$/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).

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