Therapy for chronic hepatitis C and the response

Since 2001, the standard of care for patients with chronic hepatitis C has been the combination of pegylated interferon (PEG-IFN) and ribavirin (RBV).[3,4] This combination has produced sustained virologic response (SVR) rates of 50% to 60% in patients infected with hepatitis C virus (HCV) genotype 1 who adhere to the recommended therapeutic regimen, and 40% in intention-to-treat populations, as defined by an HCV RNA negative after 6 months of completing therapy (SVR). Transient viral response (TVR) is defined as re-appearance of HCV RNA in serum after treatment has been discontinued in a patient who had undetectable HCV RNA during the therapy or on completion of the therapy (Fig. 1).[3,4] In all, only about 65% of patients become HCV RNA-undetectable when treated with this regimen;[3–5] the remaining one-third of all treated patients are classified as nonresponders (NR). Some of these patients have relatively mild liver disease but may have symptoms of HCV viremia, while others have advanced fibrosis and are at risk for developing liver complications, including decompensated cirrhosis and hepatocellular carcinoma, and the requirement for liver transplantation. Current therapies are limited by expense, ineffectiveness in a relatively high proportion of patients, numerous side effects, some of which are severe or which cause dose reduction and/or premature termination of treatment. Additionally, 10–14% of patients withdraw from IFN-based therapy prematurely,[6] with consequent high rate of treatment failure.

Spontaneous viral clearance following acute HCV infection is unusual, and the reasons for this remain unclear. Previous studies have reported that 50–85% of acutely infected-patients progress to chronicity. Identically, the relationships between race and spontaneous viral clearance following acute infection have been reported.[7–10] These characteristics based on ethnic types would suggest the effect of a host genetic factor on HCV infection.

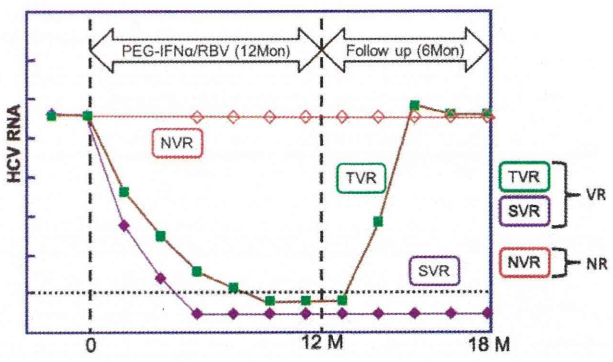

Figure 1 Representative schematic of the viral dynamics under the treatment of pegylated interferon-α (PEG-IFN-α) in combination with ribavirin (RBV). Three representative viral dynamics is observed under PEG-IFN&RBV treatment. SVR is defined as successful treatment, which is hepatitis C virus (HCV) RNA negative after 6 months of completing therapy. TVR is defined as a transiently negative of HCV RNA during treatment since HCV RNA was detected after the end of therapy. NVR is defined as a constitutive high viremia during and after treatment. NVR, non-viral response; SVR, sustained viral response; TVR, transient viral response. TVR SVR, VR; NVR, NR.

Prediction factor obtained from virus data

Predictive factors for SVR or non viral responder (NVR) as well as the prediction of severe side effects would all be highly desirable for attempts to achieve highly cost-effective therapy for patients with chronic hepatitis C. 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. Previous studies have reported viral factors associated with response to HCV treatment such as HCV genotype 1 (HCV-1), high baseline viral load, viral kinetics during treatment and amino acid pattern in the IFN sensitivity-determining region.[11–13] Accumulated data provide 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.

Host factors approached by previous strategy

Before the whole genome approach, several host or viral factors related to viral response were reported, for example, gender, age < 40 years, low HCV RNA level prior to treatment, lack of liver cirrhosis, and HCV genotypes 2/3.[14,15] For the investigation of host genome factors, genes predicted by researchers or computational prediction based on previous data have been used to determine whether the host factor, SNPs, copy number variation (CNV), or insertion/deletion of genes are related to clinical outcomes. The focused approach, however, contains the restraint to detect crucial factors because it was difficult to eliminate researcher bias in the step of candidate factor selection. 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, is able to detect strong association factors affecting disease susceptibility and drug response without any a-priori hypothesis on causative SNPs apart from the hypothesis.[16,17] Significant SNPs identified by GWAS often regulate gene expression or its function (Fig. 2). These changes affect the differences of disease phenotype or clinical response to therapy.

Whole genome association study on chronic hepatitis C

On the basis of GWAS studies, four independent groups assessed the role of genetic variation in response to PEG-IFN&RBV combination therapy for chronic hepatitis C patients, and the similar data were reported at nearly the same time.[17–20] In all cases, the conclusive finding was that polymorphisms in or near the IL28B gene strongly determined the outcome of HCV therapy. 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.[17,18] McHutchison *et al.* found genetic factors using patients from the IDEAL trial,[21] 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 Australians of Northern European ancestry with HCV genotype 1, and also validated the results

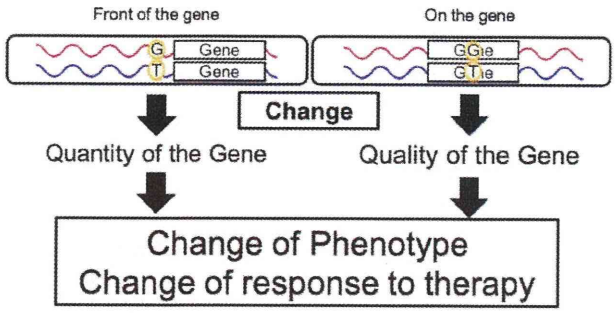

Figure 2 Single nucleotide polymorphism (SNP) function and its effect on gene against phenotype. SNPs in regulatory region or on the gene could change transcriptional activity or gene function, respectively, which affect phenotype such as outward appearance, diseases, clinical response in the case of drug treatment. However, it is not necessary that the possession of risk SNPs is directly linked to the development of diseases. Generally, almost of SNPs related with diseases have low odds ratio.

in 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 analyze host factors associated with SVR.

Tanaka *et al.* examined 142 Japanese patients with chronic hepatitis C infected with HCV genotype 1 for GWAS, and prepared an independent replication cohort of 172 Japanese (Table 1).[20] Especially, Tanaka *et al.* divided patients into three groups, SVR, TVR, or NVR, and NVR versus virological responder (VR) consisting of SVR and TVR was also used for the predication of NVR factors (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.[19] 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.

SNPs strongly associated with PEG-IFN/RBV therapy around IL-28B

Ge *et al.* identified a genetic polymorphism (rs12979860) near the *IL-28B* gene on chromosome 19, encoding IFN-λ3 (IFN-λ3). Individuals with the CC genotype showed the association with an approximately twofold better 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 ($P = 2.06 \times 10^{-3}$). Both Suppiah *et al.* and Tanaka *et al.* revealed the most significant SNPs, rs8099917 (8 kb upstream of IL-28B) associated with SVR in patients of European and Japanese. Suppiah *et al.* also identified the association of rs8099917 in European ancestry with HCV genotype 1 based on the determination of SVR factors (combined $P = 9.25 \times 10^{-9}$, odds ratio [OR] = 1.98, 95% confidence interval [CI] = 1.57–2.52) (Table 1).[17] The population with risk allele rs8099917 showed low levels of *IL-28A/B* mRNA by real-time polymerase chain reaction (PCR).[17,20]

Rauch *et al.* involved patients infected with HCV genotypes 1, 2, 3, or 4. They also identified several SNPs around the *IL-28B* gene on chromosome 19.[19] The strongest association with treatment failure was found with rs8099917 ($P = 5.47 \times 10^{-8}$; 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 viral clearance of HCV genotype 2 or 3 would be low because HCV genotype 2 or 3 is likely to be eliminated by the standard therapy (SVR 65–80%) compared with genotype 1 (SVR 40–50%).

Interestingly, Tanaka *et al.* analyzed SNPs significantly associated with NVR but not SVR. The results 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 and OR = 27.1, 95% CI = 14.6–50.3, respectively)[20] because the minor allele of the SNPs were accumulated in NVR (minor allele frequency of NVR = 74.3% for rs12980275; 75.0% for rs8099917). These data could suggest that this risk factor predicts NVR.

Spontaneous clearance of HCV infection

Rauch *et al.* and Thomas *et al.* have examined the host genetic factor(s) associated with spontaneous clearance of HCV by GWAS and candidate gene analysis, respectively.[16,19] Rauch *et al.* designed a case-control study for 347 individuals with spontaneous HCV clearance, and compared results with 567 individuals with chronic hepatitis C. The significant SNPs was again rs8099917 (combined $P = 6.07 \times 10^{-9}$, OR = 2.31, 95%CI = 1.74–3.04). Thomas *et al.* 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 same 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).

IL-28B variation and the association with response to HCV antiviral therapy after liver transplantation

Although IFN-centered antiviral therapy is significantly associated with post-transplantation graft prognosis in patients infected with HCV,[22] the efficacy of the IFN therapy after orthotopic liver transplantation (OLT) is unsatisfactory[23] and the treatment is frequently accompanied by severe side effects.[24] Therefore, in addition to the development of an optimal therapeutic regimen for HCV infection after OLT, establishment of a reliable marker or set of markers to predict the sensitivity to IFN therapy is needed. Could IL28B SNPs provide such a marker?

Fukukara *et al.* analyzed 67 recipients and 41 donors to examine the impact of genetic variations around IL-28B gene, as well as genetic variations in HCV-RNA on the responsiveness to IFN/RBV therapy for recurrent hepatitis C after OLT.[25] SVR was significantly higher in recipients carrying the major homozygous allele than in those with the minor heterozygous or homozygous allele (54% *vs* 11%; $P < 0.003$) (Table 2). SVR was also significantly higher in recipients transplanted with liver grafts from donors carrying the major homozygote (44% *vs* 9%; $P < 0.025$).

Table 1 Summary of study design and the result

Study	Ge *et al.*	Suppiah *et al.*	Tanaka *et al.*	Rauch *et al.*
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 versus non-SVR	SVR versus non-SVR	SVR versus non-SVR SVR&TVR versus NVR	SVR versus non-SVR
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)

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

Table 2 Sustained virologic response (SVR) rate of pegylated interferon-α (PEG-IFN-α) and ribavirin (RBV) therapy in liver transplantation

	SVR		*P*-value
	TT	TG/GG	
Recipient	54%	11%	< 0.003
Donor	44%	9%	< 0.025

The genotype is shown based on rs8099917.

Table 3 Sustained virologic response (SVR) rate of pegylated interferon-α (PEG-IFN-α) and ribavirin (RBV) therapy in combination analysis between recipient and donor genotype

		Recipient	
		TT	TG/GG
Donor	MA	56%	10%†
	HE/mi	10%†	0%

†These data showed same value which was analyzed using joined population of recipient with TG/GG and donor with GG, and vice versa. HE, heterozygote; MA, major homozygote; mi, minor homozygote.

Statistical analysis using both recipient and donor genotype showed that SVR was highest when both donors and recipients were major-allele homozygotes (56%; $P < 0.005$) (Table 3). Conversely a lower rate SVR (10%) was observed among recipients with the major homozygote (rs8099917, TT) who were transplanted with a liver from someone with the minor heterozygote or homozygote (rs8099917, TG or GG). Similarly, SVR rate was lower in the case where the recipient with TG or GG has received the graft from the donor with a TT genotype. Finally, no recipient with a TG or GG genotype of rs8099917 achieved SVR after they had been transplanted with liver graft from donors with TG or GG genotype of rs8099917.

Achievement of ETR was also significantly associated with SNPs around the IL-28B gene. These findings indicate that IL-28B SNPs of both the recipients and the donors influence the response to PEG-IFN and RBV therapy after OLT. These genetic variations were also significantly associated with IL-28 mRNA expression in both the resected liver derived from the recipients and in the donated liver, as reported previously.[17,20]

In summary, these studies reveal that IL-28B genetic variation in both recipients and donors is associated with IFN sensitivity of HCV infection after OLT. By using a combination of genetic analyses, the efficacy of the post-transplantation PEG-IFN and RBV therapy can now be predicted before OLT. Characterization of IL-28B SNPs in both recipient and donor with HCV-RNA may be a reliable predictor of IFN efficacy in patients with recurrent hepatitis C after OLT.

IL-28B and its function against HCV

The *IL-28B* gene has been recently discovered and classified into type III IFN that is a member of the class II cytokine family.[26,27] *IL-28B*, referred to as IFN-λ3, belongs to IFN-λ family, which consists of *IL-29*/IFN-λ1 and *IL-28A*/IFN-λ2, and *IL-28B*. IFN-λs are mainly produced by peripheral blood mononuclear cells (PBMCs) and dendritic cells.[26,27] Its expression is induced by IFN-α, viral infection, and/or stimulation of toll-like receptors (TLRs). Antiviral effects of IFN-λs against HCV were reported before the findings by GWAS. *In vitro* treatment with IFN-α, or IFN-λ1 inhibited HCV replication at similar low concentrations.[28] Combination treatment with IFN-α and *IL-29/28A* enhanced the antiviral effect against HCV replicon synergistically.[29]

As described above, HCV replication is inhibited by the antiviral effects of IFN-λ. A pegylated IFN-λ1 has already been tried against chronic hepatitis C in phase 2 trials.[30,31] Interestingly, impressive antiviral effects (at least as good as with PegIFN-α) were observed but with fewer and less severe side effects.[31] The expression pattern of IFN-λ receptor is restricted in specific tissues. High expression levels can be observed in the pancreas, liver, prostate, or thyroid, whereas central nervous system and bone marrow show only low level expression.[26,27] These results could explain why the use of IFN-lambda seems to cause less severe toxicity than that induced by IFN-α/β.

Conclusions

Genome-wide association studies have provided unexpectedly strongly positive results about the genetic factor associated with response to HCV IFN-based antiviral therapy, as well as spontaneous clearance of HCV. These findings imply a previously unsuspected role of IL-28B in the response of humans to HCV infection. Use of tests based on SNPs around the *IL-28B* gene could improve the prediction of spontaneous HCV clearance and the response to

anti-viral treatment. However, they do not provide categorical data: thus, there is an approximately 20% difference in observed clinical responses to treatment with PEG-IFN and RBV than that predicted from genetic diagnosis.[17–20] This indicates that the response to combination PEG-IFNα/RBV therapy is not inevitably restricted by heritable factors. Eligible candidates to obtain an adequate high prediction rate are needed, such as host epigenetic, rare SNPs, or genome rearrangement. In addition, these findings could be strong evidence to enhance the development of a novel therapeutic strategy such as emerging studies with IFN-λs already reveal. Further studies of IFN-λs and the role of the SNPs should be investigated to improve positive predictive value and the SVR rate by novel medicine.

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