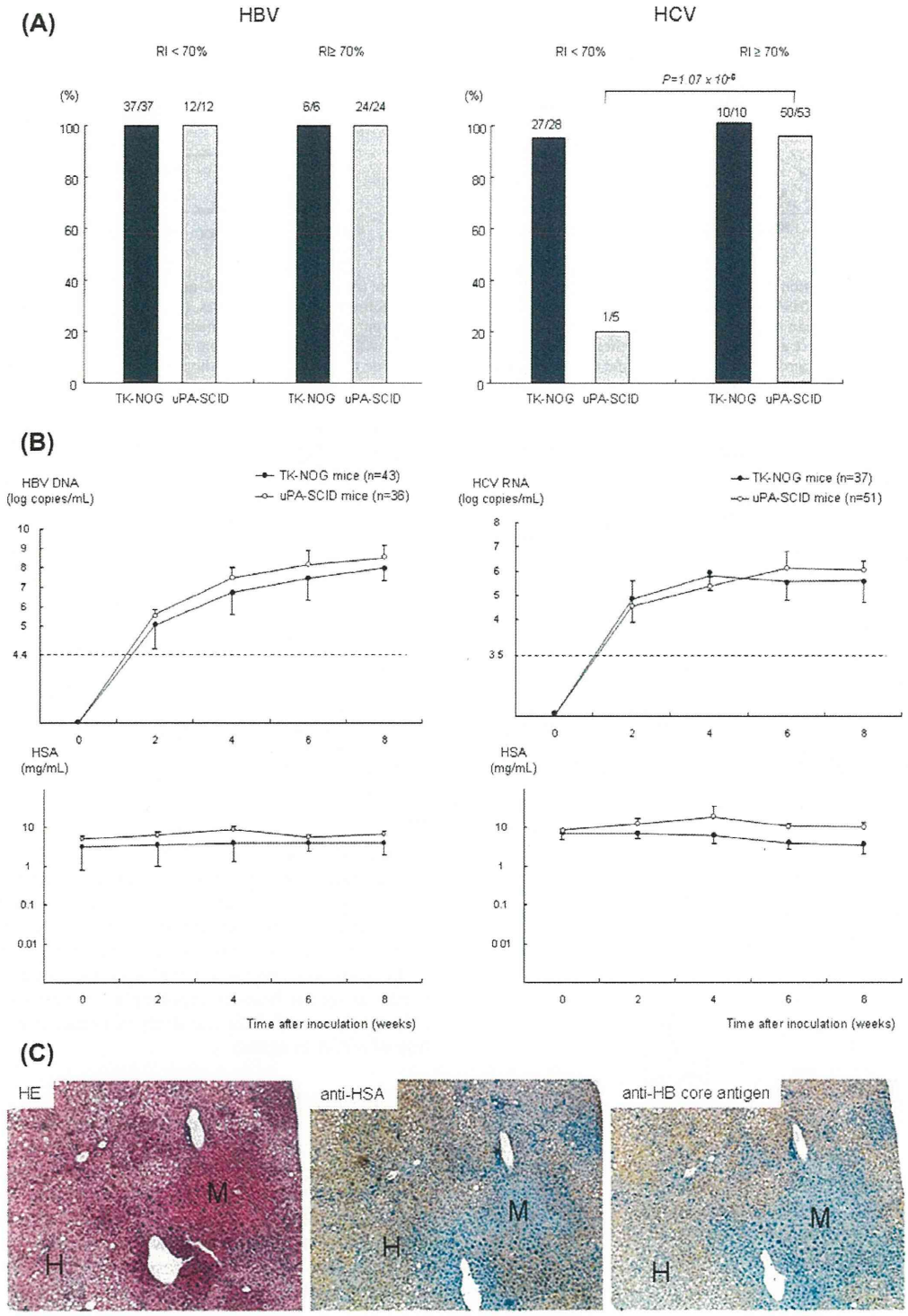

Fig. 2. Hepatitis viruses infection in chimeric mice. (A) Eight weeks after human hepatocyte transplantation, mice with serum HSA level over 1 mg/mL were inoculated with HBV- or HCV-positive human serum samples. Percentages of mice that became positive for HBV DNA (left panel) or HCV RNA (right panel) 8 weeks after inoculation according to human hepatocyte repopulation index (RI) in TK-NOG mice and uPA–SCID mice are shown. 70% of RI corresponds to 5.4 and 6.3 mg/dl of serum HAS in TK-NOG mice and uPA–SCID mice, respectively. (B) Changes in serum titers of HBV DNA (left panel) and HCV RNA (right panel) (upper panels) and HSA levels (lower panels) of TK-NOG mice and uPA–SCID mice. The horizontal dashed lines represent the lower detection limit of HBV DNA and HCV RNA (4.4 and 3.5 log copies/mL, respectively). (C) Histochemical analysis of liver samples obtained from HBV-infected TK-NOG mice. Hematoxylin-eosin staining (HE) and immunohistochemical staining using monoclonal antibodies against HSA and HB core antigen are shown. Regions are shown as human (H) and mouse (M) hepatocytes, respectively (Original magnification 100×).

reported structural differences between wild type and chimeric mice [22,23], the influence of such structural differences on HCV infectivity remains to be determined.

Human hepatocyte transplanted uPA–SCID mice are useful for evaluating antiviral agents [12–14]. In this study, we analyzed the efficacy of antiviral agents such as entecavir, IFN-alpha and

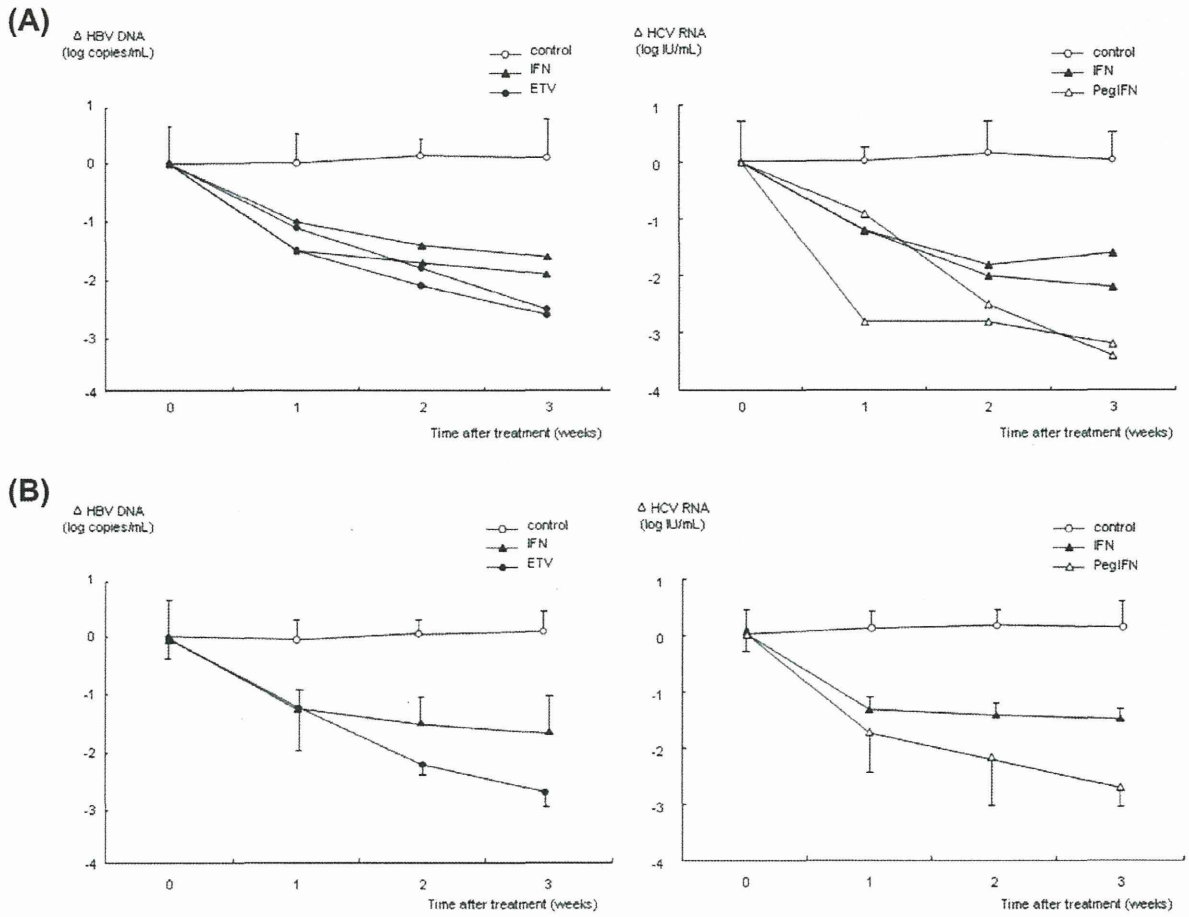

Fig. 3. Reduction of serum viral titers in mice treated with anti-viral agents. (A) HBV- (left panel) or HCV-infected (right panel) TK-NOG mice were treated with entecavir, interferon (IFN)-alpha or PegIFN-alpha-2a. Control: HBV- and HCV-infected mice without antiviral treatment. (B) HBV- (left panel) or HCV-infected (right panel) uPA–SCID mice were treated with entecavir, IFN-alpha or PegIFN-alpha-2a. Data are shown using the mean ± SD ($n = 4$).

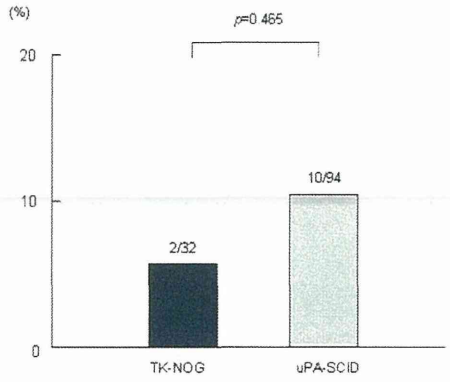

Fig. 4. Frequency of unexpected death within 8 weeks in mice. The numbers of sudden deaths occurring within 8 weeks of viral infection in TK-NOG mice and uPA–SCID mice are shown as bars.

PegIFN-alpha using HBV- and HCV-infected TK-NOG mice and compared them with uPA–SCID mice (Fig. 3). The results showed that both mouse models are equally useful for evaluation of antiviral drugs.

Human hepatocyte chimeric uPA–SCID mice are weak and prone to unexpected death [20], and this limitation appears to apply to TK-NOG mice as well. Incidence of unexpected death in the early stages of viral infection was not significantly different between TK-NOG mice and uPA–SCID mice (Fig. 4). The cause of these unexpected deaths is unknown. Further study is necessary to develop a more robust and easy to manipulate animal model.

In summary, we established a hepatitis virus infection mouse model using the human hepatocyte transplanted TK-NOG mouse. This model is useful for the study of hepatitis virology and evaluation of antiviral agents.

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Genetics of *IL28B* and HCV—response to infection and treatment

C. Nelson Hayes, Michio Imamura, Hiroshi Aikata and Kazuaki Chayama

Abstract | The *IL28B* locus attracted the attention of HCV researchers after a series of genome-wide association studies independently identified a strong association between common *IL28B* polymorphisms and the outcome of PEG-IFN-α plus ribavirin combination therapy in patients chronically infected with HCV genotype 1. This association was subsequently replicated for other HCV genotypes and has been linked to spontaneous eradication of HCV, development of steatosis and biochemical changes (such as altered levels of γ-glutamyl transpeptidase and LDL). Despite the introduction of direct-acting antiviral drugs, *IL28B* genetics are likely to play a part in patient selection and treatment decisions—moving towards a personalized approach to therapy. In HCV-infected patients with the so-called favourable *IL28B* genotype (rs12979860 CC; associated with better treatment response), hepatic expression levels of *IL28B* and interferon-stimulated genes seem to be reduced at baseline, but are induced more strongly after IFN-α administration, perhaps resulting in more effective elimination of the virus. Clarification of the mechanisms underlying these biological phenomena will lead to improved understanding of the antiviral effects of IFN-λ and, ideally, to the development of better therapies against HCV infection. This Review summarizes current understanding of the role of *IL28B* in HCV infection and response to therapy.

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Introduction

Shortly after the identification of HCV in 1989,[1] the development of HCV antibody tests—and thus improved screening and diagnosis—began to dramatically reduce the number of new cases of HCV infection by increasing awareness of this condition and by preventing transmission of HCV by blood transfusion or transplantation.[2] However, in 2011, an estimated 130–170 million people were chronically infected with HCV, putting them at an increased risk of cirrhosis, hepatocellular carcinoma and liver failure.[3] Despite the high costs and substantial toxicity of PEG-IFN-α plus ribavirin combination therapy, fewer than half of patients infected with the most common HCV genotype are able to achieve a sustained virological response (SVR),[4] which is defined as undetectable HCV RNA 6 months after the end of treatment. Although telaprevir and boceprevir are expected to greatly improve the rate of SVR, these direct-acting antiviral agents must currently be administered in combination with interferon (IFN)-α and ribavirin to help suppress viral breakthrough.[5] Identification of factors affecting response to IFN-α therapy, therefore, remains an important goal.

In 2009, a series of independent studies reported that patients infected with HCV genotype 1b who had a common variant in the *IL28B* locus (rs12979860 CC or rs8099917 TT) were significantly ($P < 7.1 \times 10^{-08}$) more likely to respond to PEG-IFN-α plus ribavirin combination therapy than patients with other *IL28B* variants.[6–8] Such patients were also more likely to spontaneously resolve acute HCV infection without treatment.[9] These results have added a new dimension to HCV research and offer the potential for more personalized and effective therapy. In the 2 years since the publication of these landmark papers, hundreds of studies have examined the role of *IL28B* polymorphisms in HCV infection and treatment. This Review summarizes some of the major findings of the role of the *IL28B* locus in HCV infection, describing background information on *IL28B* and the part IL-28B (also known as IFN-λ3) plays in the elimination of HCV and response to therapy.

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Competing interests
The authors declare no competing interests.

IL28 gene family and innate immunity

The type III IFN-λ family consists of three members: the cytokines IL-29, IL-28A and IL-28B (also known as IFN-λ1, IFN-λ2 and IFN-λ3, respectively). Although functionally an IFN, these cytokines are structurally related to the IL-10 family of cytokines.[10] Discovered in 2003 by computational prediction, the *IL28A*, *IL28B* and *IL29* genes are located in a cluster on chromosome 19 (Figure 1).[11,12] Amino acid sequences of the two isoforms of IL-28 (IL-28A and IL-28B) have 96% homology, both being 81% identical to the amino acid sequence of IL29.[12] As with IFN-α, the three IFN-λs can be triggered by viral infection and induce antiviral and antitumour activity through both innate and adaptive immune system pathways.[13,14] As shown in Figure 2a, although both IFN-α and IFN-λ family cytokines signal through

the Jak–STAT (Janus kinase–signal transducer and activator of transcription) pathway[11,15] and activate an overlapping set of IFN-stimulated genes (ISGs),[16] fundamental differences exist in gene expression between IFN-α and IFN-λ.[17] Whereas IFN-α binds to the constitutively expressed type I IFN receptor, IFN-λ cytokines bind to a heterodimer of the IL-10 and IL-28 receptors (IL10R and IL28R, respectively),[11] the latter of which is only expressed in restricted cell types, including hepatocytes, epithelial cells and plasmacytoid dendritic cells.[18] Although both types of IFN induce expression of many of the same genes, the change in ISG expression in response to IFN-λ tends to be weaker overall but increases steadily over time,[16] whereas IFN-α triggers an early peak in ISG expression followed by a rapid decline.[19] This phenomenon seems to result from the distinct kinetics of IFN-λ-mediated activation of STAT,[19] involving differences in transcription factor remodelling efficiency[16] and greater dependence on the NF-κB pathway.[20] Therefore, differences in ISG expression through type I and III IFNs are possible under specific conditions.[20]

Key points

- The 130–170 million people chronically infected with HCV have an increased risk of cirrhosis, hepatocellular carcinoma and liver failure
- Several single nucleotide polymorphisms upstream of the *IL28B* gene are associated with spontaneous clearance of HCV and improved response to PEG-IFN-α plus ribavirin combination therapy
- In patients with the so-called favourable *IL28B* allele (rs12979860 CC), associated with better response to therapy, HCV RNA levels decline rapidly with treatment and IFN-α therapy induces strong interferon-stimulated gene (ISG) expression
- In patients with unfavourable *IL28B* genotypes (rs12979860 CC/TT), ISG expression tends to be refractory to further IFN stimulation, resulting in poor response to IFN therapy
- *IL28B* genotype might also predict response to telaprevir triple therapy, although it might not be as effective at predicting the treatment response in this scenario as with PEG-IFN-α plus ribavirin combination therapy

Effects of *IL28B* polymorphisms

An inherent limitation of genome-wide association studies (GWAS; Box 1) is the difficulty in tracing the link between changes at a single base and differences in the resulting phenotype. Understanding the genetic basis of differences in resolution of HCV infection is yet more complex because it involves coordinated activity between innate and adaptive immune effectors and is affected by multiple host and viral factors. The fairly strong independent effect of *IL28B* polymorphisms therefore suggest a role in the high-level regulation of antiviral defence against HCV. The effects of *IL28B* polymorphisms have been investigated for a number of aspects of response to HCV infection and treatment, including response to therapy, natural elimination of the virus, and changes in gene expression and lipid metabolism.

PEG-IFN-α plus ribavirin combination therapy

Striking differences in HCV clearance and response to treatment among ethnic groups,[21] as well as among patients infected with the same HCV inoculum,[22] have long suggested a role for host genetic factors. Early candidate gene studies identified single nucleotide polymorphisms (SNPs) in genes encoding proteins involved in response to HCV infection—including osteopontin, MxA (also known as MX1), OAS1, EIF2AK2 (also known as PKR), IFN-α receptor 1, and MAPKAPK3[23–26]—but practical insights gained from these studies have been limited. SNPs are defined as base pair variants at a specific genomic position that have a frequency greater than 1%.[27] As the number of SNPs examined increases, the probability of detecting spurious associations also increases sharply and a drawback of candidate gene studies, in particular, is the need to select appropriate target genes prior to analysis, limiting the ability to detect novel associations. Advances in high-throughput screening, however, have made it possible to screen representative SNPs across the entire genome using GWAS. This 'hypothesis-free' approach can detect SNPs associated with disease phenotype or response to treatment without requiring *a priori* candidate gene selection. Nonetheless, most SNPs are thought to have small, cumulative effects that require large sample sizes to be detected, and results of GWAS often fail to be replicated in other populations. Although the importance of ethnicity in treatment outcome suggests a genetic basis, the simultaneous independent discovery of a common variant upstream of the *IL28B* gene and the magnitude of its effect on treatment outcome was unusual (Table 1).[6–8]

Ge *et al.*[6] published the first report of an association between a common polymorphism on chromosome

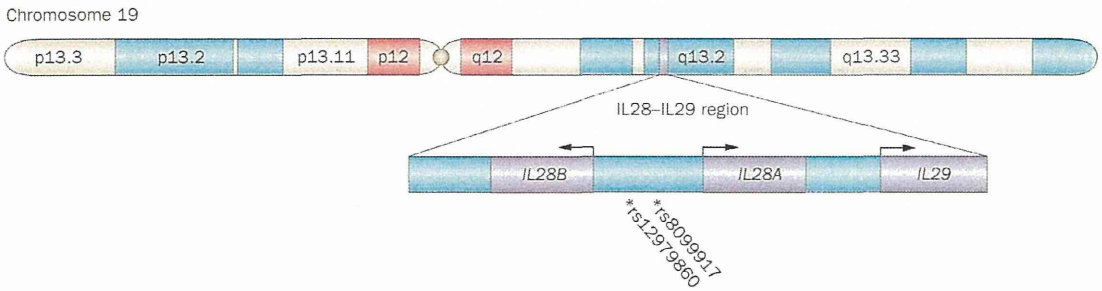

Figure 1 | The *IL28–IL29* locus on chromosome 19. The genes encoding the three members of the IFN-λ family, IL28A (IFN-λ2), IL28B (IFN-λ3) and IL29, (IFN-λ1) are clustered together on chromosome 19. The most important and validated SNPs with respect to response to IFN-α therapy, rs12979860 and rs8099917, are upstream of both *IL28B* and *IL28A* (owing to their antiparallel orientation), but are physically closer to *IL28B*.

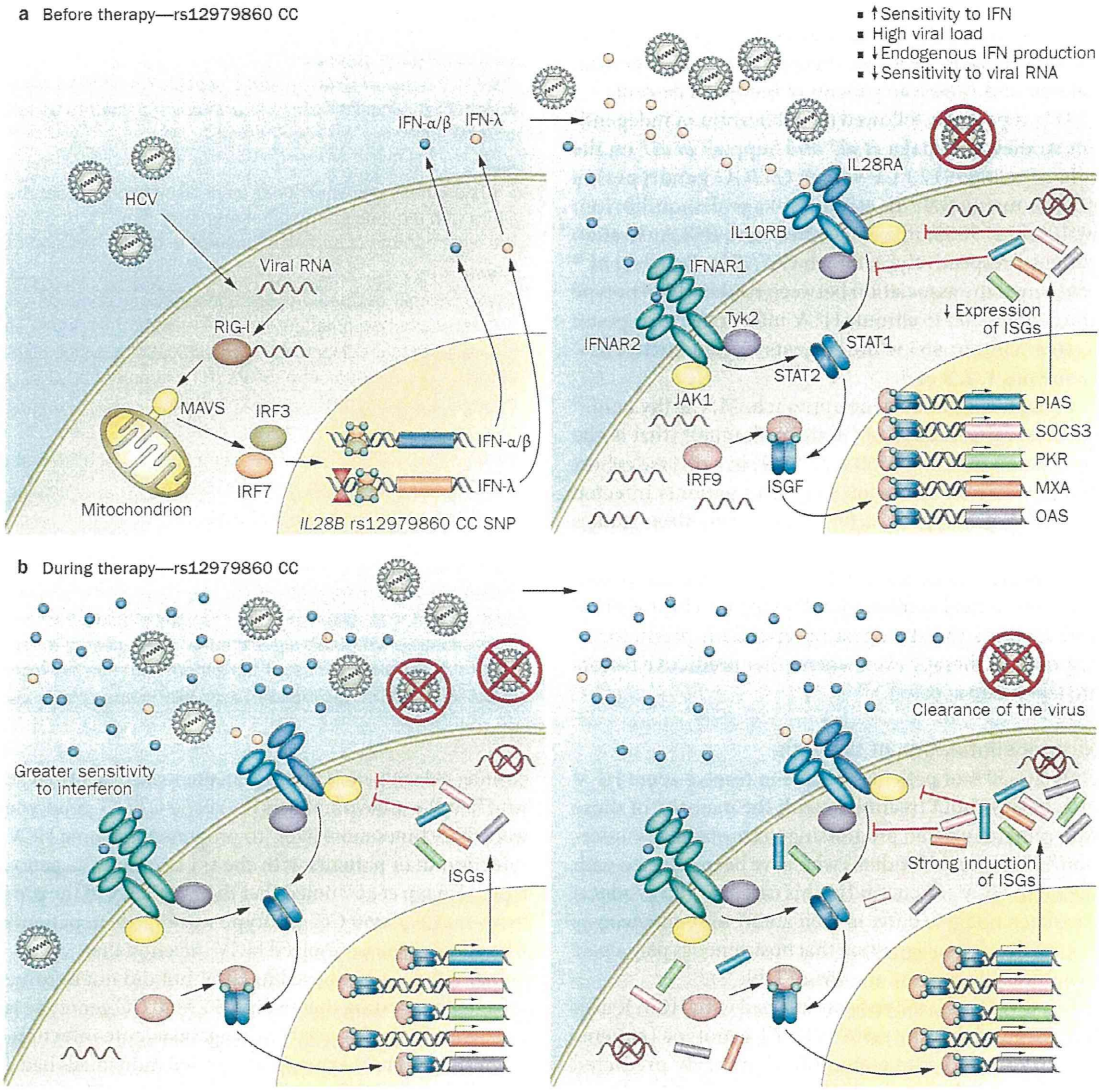

Figure 2 | Potential role of the favourable *IL28B* genotype in the response to interferon therapy. **a** | Intracellular HCV RNA is detected by surveillance molecules of the innate immune system, including the RIG-I–IFIH1 pathway, which causes the adaptor protein MAVS to induce expression and secretion of IFN-α, IFN-β and IFN-λ via IRFs. IFNs are recognized by receptors on the cell surface and initiate a signal cascade that results in induction of a large number of ISGs that collectively establish an antiviral state hostile to viral replication. IFN-α and IFN-β are recognized by IFNAR, whereas IFN-λ is recognized by the IL10R–IL28R receptor complex. Both receptors activate the Jak-STAT pathway, which induces the translocation of an ISGF complex to the nucleus, where it binds to the interferon-stimulated response elements of multiple ISGs. In individuals with the favourable allele (rs12979860 CC), the presence of viral RNA seems to induce only weak expression of IFN-λ, which in turn induces weak ISG expression. Although the response might be sufficient to clear the virus at low viral loads, the cell seems fairly tolerant of the virus and high viral loads can accumulate. **b** | During treatment, however, when IFN-α is administered, IFN signal transduction is unimpeded and results in strong ISG induction. As a result, these patients tend to respond well to therapy and are able to clear the virus efficiently. Abbreviations: IFIH1, IFN-induced helicase C domain-containing protein 1; IFNAR, IFN-α/β receptor 1; IL10R, IL-10 receptor; IL28R, IL-28 receptor; IRF, IFN regulatory factor; ISG, IFN-stimulated gene; ISGF, IFN-stimulated gene factor; Jak, Janus kinase; MAVS, mitochondrial antiviral-signalling protein; MXA, myxovirus resistance protein 1 (also known as MX1); OAS, 2'5'-oligoadenylate synthase; PIAS, E3 SUMO-protein ligase PIAS; PKR, protein kinase RNA-activated (also known as EIF2AK2); RIG-I, retinoic-acid inducible protein I; SOCS3, suppressor of cytokine signaling 3; STAT, signal transducer and activator of transcription.

19 and SVR on the basis of an analysis of a large cohort of white, African American and Hispanic patients with chronic HCV genotype 1 infection who were treated with 48 weeks of combination therapy with PEG-IFN-α plus ribavirin.[6] The rs12979860 SNP identified in this study is located in a noncoding region 3 kb upstream of *IL28B*. Patients homozygous for the major allele with the so-called favourable genotype (rs12979860 CC) were twice as likely to achieve an SVR as patients with the minor risk allele (rs12979860 TT or CT) following combination therapy.

The frequency of the major allele was proportional to the SVR rate across ethnic groups and explained half of the difference in SVR rates between African-American patients and American patients of European descent.

This report was followed by publication of independent studies by Tanaka *et al.*[7] and Suppiah *et al.*[8] on the role of rs8099917 (TT versus GT/GG genotypes), a neighbouring SNP in strong linkage disequilibrium with rs12979860, in 314 Japanese and 848 Australian patients, respectively. A fourth GWAS by Rauch *et al.*[28] confirmed the association between rs8099917 genotype and progression to chronic HCV infection and response to treatment in 465 white patients infected with HCV genotypes 1, 2, 3 or 4.

Using a candidate gene approach, McCarthy *et al.*[29] replicated the findings of Ge and colleagues[6] (that is, the association of rs12979860 with SVR) in a diverse cohort of 231 white and African-American patients infected with HCV genotypes 1, 2, or 3. Collectively, these studies suggest that either the rs12979860 CC or the rs8099917 TT genotype confers a 2–3-fold improved likelihood of response to dual combination therapy for chronic HCV and remains the strongest independent predictor of response to therapy even when other predictive factors are taken into account.[6,28]

Box 1 | Glossary terms for genome-wide studies

Candidate gene approach
Prior to the use of GWAS, genetic association studies were often performed by sequencing candidate genes known or suspected to be involved in with a disease or condition.

SNP
SNPs are base pair variants at a specific genomic location. Typically, only common SNPs with a population frequency greater than 1% or 5% are considered, due to the large number of samples required to detect associations involving rare SNPs.

GWAS
GWAS typically examine associations between common single nucleotide differences between patients in a disease group (cases) and a healthy group (controls) or other dichotomous classifications. Using high-throughput array-based methods, a large number of SNPs (for example, 500,000 or 1 million) can be assayed simultaneously for each patient.

Linkage disequilibrium
Linkage disequilibrium refers to combinations of alleles at different loci that occur together more often than expected by chance.

Causative SNP versus tagging SNPs
Due to linkage disequilibrium, GWAS can detect associations between a response variable and multiple SNPs in a region of DNA. In many cases, only one or a small number of SNPs directly affect the phenotype (for example, by changing an amino acid or altering transcription factor binding affinity). SNP arrays are based on a representative set of tagging SNPs that are expected to fall within linkage blocks that collectively cover most of the genome but may not include the causative SNPs.

Abbreviations: GWAS, genome-wide association studies; SNP, single nucleotide polymorphism.

Natural elimination of the virus

Only 20–30% of patients are able to resolve acute HCV infection without treatment, with the majority of those who remain infected progressing to chronic HCV infection.[9] However, individuals who have been infected with the same HCV inoculum (in this case, tainted product) have been noted to differ in their ability to spontaneously resolve the virus, suggesting that host genetics play a part in natural clearance of the virus (Table 2).[22]

In a GWAS of 1,362 patients infected with HCV, Rauch *et al.*[28] found that the rs8099917 TT genotype (patients homozygous for the major allele) strongly predicted spontaneous clearance of HCV infection. Thomas and colleagues[9] examined whether the SNP identified in the Ge *et al.*[6] study (rs12978860) was also associated with spontaneous clearance. They genotyped 1,008 patients with acute HCV infection and found that those with the rs12979860 CC genotype were more likely to spontaneously clear the virus than those with rs12979860 CT or TT genotype.[9] Tillmann *et al.*[30] also confirmed these results in a study of 136 German women, in whom spontaneous clearance occurred more often in patients with the CC genotype than either non-CC (CT or TT) genotype. Patients with a non-CC genotype were more likely to spontaneously clear the virus when they developed jaundice, but jaundice was not associated with viral clearance in patients with the CC genotype.[30]

Ruiz-Extremera and colleagues[31] examined the role of *IL28B* polymorphisms on vertical transmission of HCV between mother and child and found that whereas *IL28B* genotype in the mother and child are unrelated to vertical transmission, children with the rs12979860 CC genotype were more likely to spontaneously clear HCV genotype 1 infection. In a study of 138 Brazilian patients co-infected with HCV and HIV, patients with acute HCV who had the unfavourable rs12979860 CT/TT genotype were three times more likely to progress to chronic HCV infection than patients with the rs12979860 CC genotype.[32] Knapp *et al.*[33] noted that the frequency of the protective rs12979860 CC genotype was higher in patients who spontaneously resolved HCV infection than in individuals who were exposed to HCV but did not become infected, suggesting that the rs12979860 CC genotype is not associated with protection against acute infection. Interestingly, HCV-exposed uninfected individuals had a high frequency of killer cell immunoglobulin-like receptor 2DL3:group 1 HLA-C (KIR2DL3:HLA-C1),[33] which highlights the importance of the innate immune system in antiviral defence and suggests other heritable factors might be involved.

Even though high viral load is associated with poor response to treatment,[34] multiple studies have reported an association between high viral load and the favourable *IL28B* genotype (rs12979860 CC).[6,29,35–37] One potential explanation for this phenomenon is that patients with the favourable allele might be able to spontaneously clear the virus when the viral load is below a certain threshold. As a result, patients with the favourable *IL28B* genotype presenting with low viral loads might be relatively uncommon. Understanding how an individual SNP enables some patients to efficiently clear the virus in the absence of therapy should yield insight into the regulation of antiviral defences.

Change in viral load

Although the *IL28B* SNP remains the strongest pretreatment predictor of SVR,[38] on-treatment predictors such

Table 1 | Associations between *IL28B* homozygous major allele and response to HCV therapy

Study	SNP	Total number of patients	Odds ratio	*P* value	Population	HCV genotype
SVR with PEG-IFN-α plus ribavirin combination therapy						
Ge *et al.* (2009)[6]	rs12979860 CC	1,137	3.10	1.21×10^{-28}	White, African American, Hispanic	1
Suppiah *et al.* (2009)[8]	rs8099917	293	1.98	7.06×10^{-8}	White	1
Tanaka *et al.* (2009)[7]	rs8099917	142	12.10	3.11×10^{-15}	Japanese	1
Rauch *et al.* (2010)[28]	rs8099917	465	5.20	5.47×10^{-8}	Swiss	1–4
McCarthy *et al.* (2010)[29]	rs12979860 CC	231	5.80	9.00×10^{-6}	White, African American	1–3
Thompson *et al.* (2010)[38]	rs12979860 CC	1,671	5.20	$<1.00\times10^{-4}$	White, African American, Hispanic	1
Ochi *et al.* (2011)[37]	rs8099917	594	2.46	6.52×10^{-8}	Japanese, Taiwanese	1, 2
SVR with telaprevir triple therapy						
Akuta *et al.* (2010)[96]	rs8099917	66	10.60	$<1.00\times10^{-3}$	Japanese	1
Chayama *et al.* (2011)[70]	rs8099917	94	8.33	1.40×10^{-2}	Japanese	1
SVR with combination therapy for non-1b HCV genotypes*						
Mangia *et al.* (2010)[63]	rs12979860 CC	268	1.76	1.13×10^{-6}	White	2, 3
Asselah *et al.* (2011)[64]	rs12979860 CC	164	3.32	8.00×10^{-4}	Egyptian, European, sub-Saharan African	4
Kawaoka *et al.* (2011)[65]	rs8099917	83	4.35	2.00×10^{-2}	Japanese	2
Lindh *et al.* 2011[66]	rs12979860 CC	341	NA	2.00×10^{-2}	White	2, 3
Sakamoto *et al.* (2011)[67]	rs8099917	129	3.96	1.04×10^{-1}	Japanese	2
Sarrazin *et al.* (2011)[68]	rs12979860 CC	267	2.80	9.00×10^{-3}	German population	2, 3

Representative studies are shown, along with results based on the most significant SNP (rs12979860 or rs8099917), sample size, *P* value, odds ratio with respect to the favourable allele (if reported), study population, and viral genotype. *Initial *IL28B* studies focused mainly on the difficult-to-treat genotype 1 with 48 weeks of PEG-IFN-α plus ribavirin combination therapy. Other genotypes may respond better to therapy, and treatment guidelines may differ slightly with respect to stopping rules and overall duration of therapy.[97] Abbreviations: NA, not available; SVR, sustained virological response.

as rapid virological response (RVR; that is, undetectable HCV RNA by week 4 of therapy) are more directly linked to treatment outcome (Table 2).[39,40] When the effect of RVR is taken into account, *IL28B* genotype might no longer be an important predictor of treatment outcome,[41] especially during infection with HCV genotypes other than 1b.[42] However, *IL28B* genotype influences on-treatment predictors in several ways. On the one hand, initial baseline viral load tends to be higher in patients with the favourable *IL28B* genotype.[6,34–37] On the other hand, these patients seem to clear the virus more efficiently at each time point examined (for example, 48 h,[43] 4 weeks,[34] 12 weeks,[44] and so on). Consequently, patients with the favourable *IL28B* genotype are more likely to achieve RVR,[38,39,45] and even among patients who fail to achieve RVR, *IL28B* genotype remains the strongest predictor of SVR.[38,44] *IL28B* genotype might be linked to higher death rates of infected hepatocytes in patients with the favourable genotype[43] as well as lower daily viral production rates,[46] which could partially explain the improved efficiency of virological response in those with the *IL28B* CC genotype compared with non-CC *IL28B* genotypes.

ISG expression and viral replication

Both IFN-α and IFN-λ induce expression of hundreds of target ISGs and, subsequently, an antiviral state. Consequently, ISG expression might be the best predictor of treatment response regardless of *IL28B* genotype,[47] although genotyping one or a few *IL28B* SNPs is probably more practical than assaying ISG expression levels. Unexpectedly, however, hepatic ISG expression was found to be markedly higher in patients with the unfavourable *IL28B* allele,[48] and patients with high baseline ISG expression levels were found to respond poorly to IFN-α therapy.[49] Hepatic expression of several ISGs—including *MXA* (also known as *MX1*), *EIF2AK2*, *OAS1* and *ISG15*—was lower in patients with the favourable *IL28B* genotype than in those with the unfavourable *IL28B* genotype;[50] conversely, expression of genes that suppress the antiviral state were reduced.[51] However, Shebl *et al.*[52] found no evidence of an association between *IL28B* genotype and ISG expression in hepatocytes from uninfected individuals, implying that the association between *IL28B* genotype and ISG expression in the liver does not reflect normal expression levels in healthy individuals.[52] HCV infection in nonresponders might result in continual, but ineffectual, intrahepatic ISG expression, including expression of IFN-signalling inhibitors. Cells with such preactivated ISGs might not only fail to effectively clear the virus, but might have reduced sensitivity to therapeutic IFN-α (Figure 3).[49,53]

Biochemical changes and hepatic steatosis

During chronic HCV infection, differences in the cytokine profiles induced by the *IL28* polymorphisms

Table 2 | Associations between *IL28B* homozygous major allele and host response to HCV

Study	SNP	Total number of patients	Odds ratio	*P* value	Population	HCV genotype
Spontaneous clearance of HCV infection						
Thomas *et al.* (2009)[9]	rs12979860 CC	1,008	3.03	$<1.00\times10^{-12}$	European and African ancestry	1
Grebely *et al.* (2010)[71]	rs8099917 TT	163	3.78	4.40×10^{-2}	Australian	1–3
Montes-Cano *et al.* (2010)[72]	rs12979860 CC	731	3.13	6.20×10^{-5}	Spanish	NA
Tillmann *et al.* (2010)[30]	rs12979860 CC	136	NA	$<1.00\times10^{-3}$	German women	1b
Knapp *et al.* (2011)[33]	rs12979860 CC	397	2.97	1.00×10^{-4}	UK	NA
Rauch *et al.* (2010)[28]	rs8099917 TT	1,362	2.31	6.07×10^{-9}	Swiss	1–4
Improved rapid and early viral dynamics (RVR, EVR)						
Thompson *et al.* 2010[38]	rs12979860 CC	1,671	NA	$<1.00\times10^{-4}$	White, African American, Hispanic	1
Bochud *et al.* (2011)[41]	rs12979860 CC	242	NA	7.00×10^{-3}	White	1–3
Hayes *et al.* (2011)[35]	rs12979860 CC	817	1.37	1.40×10^{-8}	Japanese	1
Lin *et al.* (2011)[44]	rs12979860 CC	191	NA	$<1.00\times10^{-3}$	Taiwanese	1
Stattermayer *et al.* (2011)[45]	rs12979860 CC	682	NA	$<1.00\times10^{-3}$	Austrian	1–4
Ochi *et al.* (2011)[37]	rs8099917 TT	594	NA	6.70×10^{-5}	Japanese, Taiwanese	1, 2
Increased baseline viral load						
Ge *et al.* (2009)[6]	rs12979860 CC	1,475	NA	1.20×10^{-10}	White, African American, Hispanic	1
McCarthy *et al.* (2010)[29]	rs12979860 CC	231	2.13	6.10×10^{-3}	White, African American	1–3
Lindh *et al.* (2011)[66]	rs12979860 CC	341	NA	$<1.00\times10^{-3}$	White	2, 3
Ochi *et al.* (2011)[37]	rs8099917 TT	594	2.46	1.00×10^{-2}	Japanese, Taiwanese	1, 2
Increased viral clearance rate and death rates among infected hepatocytes						
Hsu *et al.* (2011)[46]	rs8099917 TT	145	NA	$<2.34\times10^{-2}$	Taiwanese	1, 2
Scott *et al.* (2011)[43]	rs12979860 CC	20	NA	4.00×10^{-2}	White, African American	1, 3

Representative studies are shown, along with results based on the most significant SNP (rs12979860 or rs8099917), sample size, *P* value, odds ratio with respect to the favorable allele (if reported), study population, and viral genotype. Abbreviations: EVR, early virological; NA, not available; RVR, rapid virological response; SVR, sustained virological response.

lead to altered biochemical and inflammatory states (Table 3).[54] Patients with an unfavorable *IL28B* genotype have been shown to have an increased risk of HCV-associated cirrhosis and more severe fibrosis.[55,56] However, Marabita *et al.*[57] reported that when the date of infection is taken into account, *IL28B* genotype is no longer associated with rate of fibrosis progression.

Lipid metabolism has an important role in HCV infection, and patients with high cholesterol levels tend to respond better to IFN therapy than those with lower cholesterol levels.[58] However, HCV infection and IFN administration both tend to depress cholesterol levels. Patients with the favourable *IL28B* genotype tend to have higher levels of total cholesterol, apolipoprotein B and LDL-cholesterol,[58] as well as a lower frequency of hepatic steatosis, than patients with the unfavourable *IL28B* genotype.[59]

Identification of the causative SNP

Many treatment-associated SNPs in the *IL28B* locus have been reported, although most studies have examined either rs12979860 or rs8099917.[28] Although rs12979860 seems to have better predictive value in some populations,[60] haplotypes that include both SNPs might be more accurate than either SNP individually.[61] This finding suggests that neither SNP is directly causative, but that both are linked to one, or more, as-yet-unidentified causative SNPs. Linkage disequilibrium (Box 1) is lowest in the African-American population, suggesting that examination of this group offers the best chance to identify the causative SNP.[6,62] Massively parallel sequencing has been used to identify new *IL28B* variants and Smith and colleagues[62] reported two SNPs—rs4803221 and rs7248668—that are more strongly associated with treatment failure than rs8099917. The authors do, however, point out that the high degree of homology among *IL28B* and *IL28A* complicates the task of unambiguous read mapping and increases the risk of detecting spurious associations.[62]

Although SNPs typically have little individual effect on treatment outcome, the minor allele (rs12979860 CT/TT) in the *IL28B* locus increases the risk of treatment failure 2–3-fold (observation based on references in Table 1), suggesting a direct effect on gene expression

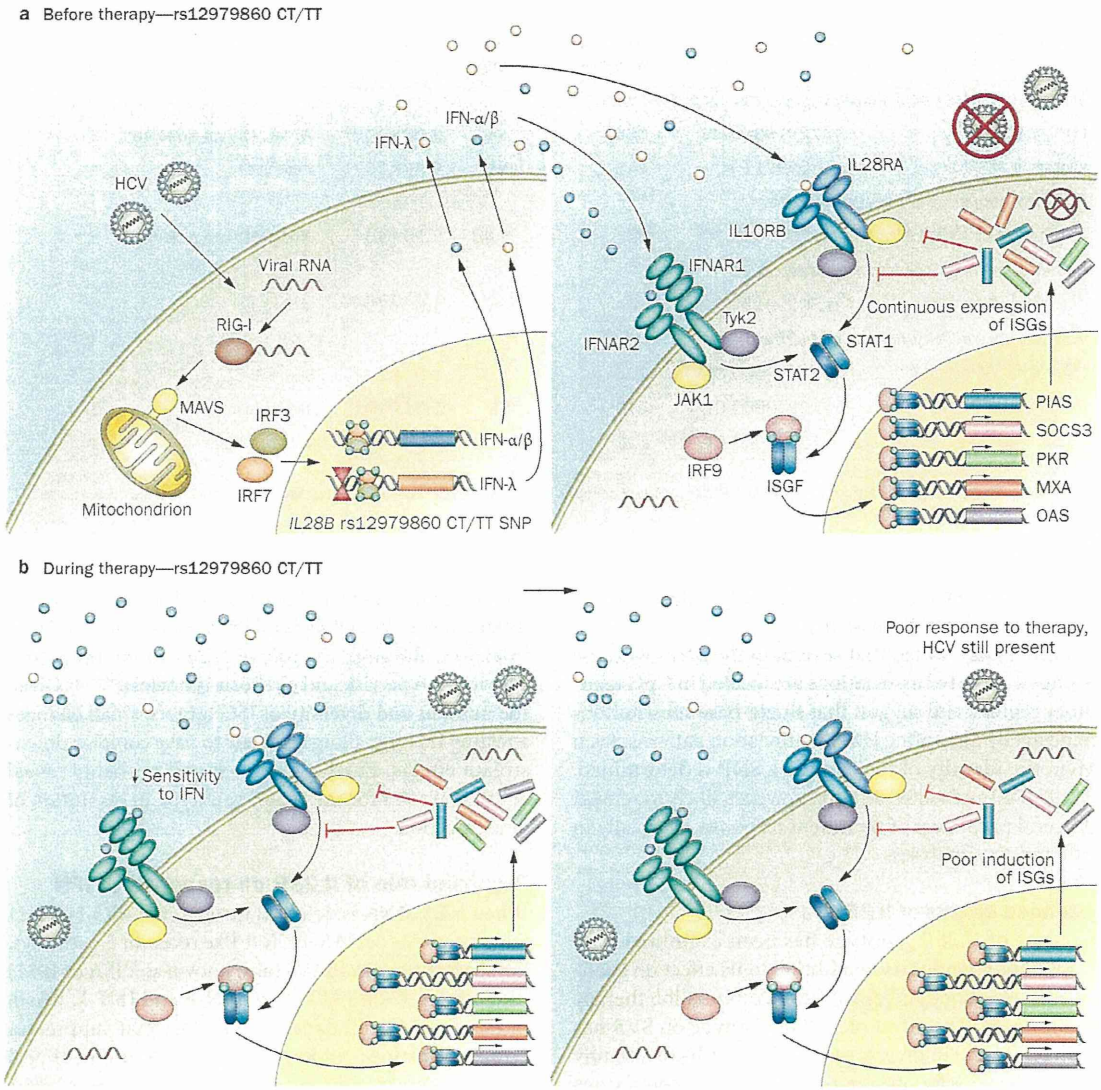

Figure 3 | Potential role of the unfavourable *IL28B* genotype in the response to interferon therapy. **a** | Intracellular HCV RNA is detected by surveillance molecules of the innate immune system, including the RIG-I–IFIH1 pathway, which causes the adaptor protein MAVS to induce expression and secretion of IFN-α, IFN-β and IFN-λ via IRFs. IFNs are recognized by receptors on the cell surface and initiate a signal cascade that results in induction of a large number of ISGs that collectively establish an antiviral state hostile to viral replication. IFN-α and IFN-β are recognized by IFNAR, whereas IFN-λ is recognized by the IL10R–IL28R receptor complex. Both receptors activate the Jak-STAT pathway, which induces the translocation of an ISGF complex to the nucleus, where it binds to the interferon-stimulated response elements of multiple ISGs. Unlike patients with the favourable *IL28B* (rs1297860 CC) genotype, in which viral RNA induces only modest ISG expression, patients with the unfavourable genotype (rs12979860 CT/TT) tend to have higher baseline ISG expression levels, suggesting continuous stimulation of the IFN signaling pathway in the presence of the virus. Nonetheless, the level of ISG expression seems to be insufficient to clear the virus, at the same time triggering negative regulation of the IFN signaling pathway through IFN-inhibitory molecules such as SOCS3 and PIAS. These pathways negatively regulate Jak-STAT signaling and make the cell less responsive to IFN signaling. **b** | As a result, even when IFN-α is administered as part of therapy, the cell is unable to induce a strong ISG expression, and the patient responds poorly to therapy. Abbreviations: IFIH1, IFN-induced helicase C domain-containing protein 1; IFNAR, IFN-α/β receptor 1; IL10R, IL-10 receptor; IL28R, IL-28 receptor; IRF, IFN regulatory factor; ISG, IFN-stimulated gene; ISGF, IFN-stimulated gene factor; Jak, Janus kinase; MAVS, mitochondrial antiviral-signalling protein; MXA, myxovirus resistance protein 1 (also known as MX1); OAS, 2'5'-oligoadenylate synthase; PIAS, E3 SUMO-protein ligase PIAS; PKR, protein kinase RNA-activated (also known as EIF2AK2); RIG-I, retinoic-acid inducible protein I; SOCS3, suppressor of cytokine signaling 3; STAT, signal transducer and activator of transcription.

resulting from a single base change. A number of possible mechanisms exist, and so far little evidence is available to reject some of the possibilities. The causative *IL28B* SNP might affect the strength, timing or specificity of *IL28B* expression by altering binding of transcription factors, enhancers or other regulatory elements, or it