

# Host – hepatitis C viral interactions: The role of genetics

Markus H. Heim<sup>1,2,\*</sup>, Pierre-Yves Bochud<sup>3,\*</sup>, Jacob George<sup>4,\*</sup>

**Keywords:** IL28B; GWAS; Interferon; Hepatitis C virus; Innate immunity; Jak-STAT; CD8<sup>+</sup> T cells; T cell exhaustion; Viral escape; Fibrosis; Hepatocellular cancer; Haplotypes.

Received 29 June 2016; received in revised form 29 July 2016; accepted 29 July 2016

<sup>1</sup>Division of Gastroenterology and Hepatology, University Hospital Basel, Petersgraben 4, 4031 Basel, Switzerland;

<sup>2</sup>Department of Biomedicine, University of Basel, Hebelstrasse 20, 4031 Basel, Switzerland;

<sup>3</sup>Infectious Diseases Service, University Hospital and University of Lausanne, Rue du Bugnon 46, 1011 Lausanne-CHUV, Switzerland;

<sup>4</sup>Storr Liver Centre, Westmead Institute for Medical Research, Westmead Hospital and University of Sydney, NSW, Australia

## Summary

Hepatitis C virus (HCV) is a major cause of chronic viral hepatitis that can lead to cirrhosis and hepatocellular carcinoma. Only a minority of patients can clear the virus spontaneously. Elimination of HCV during acute infection correlates with a rapid induction of innate, especially interferon (IFN)-induced genes, and a delayed induction of adaptive immune responses. There is a strong association between genetic variants in the *IFNλ* (*IL28B*) locus with the rate of spontaneous clearance. Individuals with the ancestral *IFNλ4* allele capable of producing a fully active *IFNλ4* are paradoxically not able to clear HCV in the acute phase and develop chronic hepatitis C (CHC) with more than 90% probability. In the chronic phase of HCV infection, the wild-type *IFNλ4* genotype is strongly associated with an induction of hundreds of classical type I/type III IFN stimulated genes in hepatocytes. However, the activation of the endogenous IFN system in the liver is ineffective in clearing HCV, and is even associated with impaired therapeutic responses to pegylated (Peg)IFNα containing treatments. While the role of genetic variation in the *IFNλ* locus to the outcome of CHC treatment has declined, it is clear that variation not only at this locus, but also at other loci, modulate clinically important liver phenotypes, including inflammation, fibrosis progression and the development of hepatocellular cancer.

In this review, we summarize current knowledge about the role of genetics in the host response to viral hepatitis and the potential future evolution of knowledge in understanding host-viral interactions.

© 2016 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.

## Key point

Genetic variants of the interleukin 28B (*IL28B*) gene locus are strongly associated with spontaneous clearance of HCV and with response to therapy with pegylated IFN-α and ribavirin.

**Abbreviations:** AHC, acute hepatitis C; CHC, chronic hepatitis C; CNV, copy number variant; CRS, cirrhosis risk score; DC, dendritic cell; EWAS, epigenome-wide association study; GWAS, genome wide association study; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular cancer; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; IFN, interferon; IFNAR,

## Introduction

Hepatitis C virus (HCV) is parenterally transmitted, mainly due to injection drug use and unsafe transfusions and therapeutic injections [1]. Acute HCV infections are often oligo- or asymptomatic [2]. In 60–80% of those infected, the virus persists and the infection becomes chronic. Spontaneous clearance of HCV is rare in the chronic phase of the infection [3]. In most patients, chronic hepatitis C (CHC) leads to some degree of liver fibrosis; cirrhosis develops in 11–37% of patients, after 20 years in tertiary hospital and post-transfusion cohorts and in 4–10% of community-based cohorts [4]. A systematic review suggested that the estimated prevalence of cirrhosis at 20 years after infection was 16% (14–19%) for all included studies, varying from 18% (16–21%) for studies in clinical settings to 7% (4–12%) for studies in non-clinical settings [5]. Patients with CHC and cirrhosis are at increased risk for liver failure and for developing hepatocellular carcinoma [6]. Genome wide association studies

(GWAS) revealed a strong association of genetic variants near the interleukin 28B (*IL28B*) gene with spontaneous and pegylated interferon alpha (PegIFNα) treatment-induced clearance of HCV [7–12]. *IL28B* corresponds to interferon (IFN)λ3, and more recently it has become clear that the functional single nucleotide polymorphisms (SNPs) are in the newly discovered *IFNλ4* gene that is located next to *IFNλ3* (*IL28B*) on human chromosome 19q13 [13]. In 2016, with the increasing availability of, and access to highly effective antiviral regimens for the treatment of CHC, the relevance of host genetic variation to the outcome of treatment is diminishing. However, based on the foundations of the landmark genetic discoveries concerning *IFNλ4*, recent studies have highlighted the importance of genetic variation to other liver disease phenotypes during the evolution of viral hepatitis, including liver inflammation, steatosis, fibrosis and hepatocellular carcinoma (HCC).

### Host-virus interactions in acute hepatitis C

Host-virus interactions in the acute phase of HCV infections have been studied mainly in experimentally infected chimpanzees [14–17]. HCV has an enormous replicative capacity and reaches high serum titers of  $10^5$ – $10^7$  IU/ml already within days after infection. In the early phase of acute hepatitis C (AHC), the host reacts with an equally rapid IFN response (Fig. 1). The induction of hundreds of IFN stimulated genes (ISGs) by type I (IFN $\alpha$ s/IFN $\beta$ ) and/or type III IFNs (IFN $\lambda$ s) probably restricts viral replication to some extent, but for unknown reasons fails to eliminate HCV. After a delay of 4–8 weeks, HCV specific T cells are recruited to the liver in the second or late phase of AHC. This phase lasts 4–10 weeks and is a unique window of opportunity for the immune system to eliminate HCV infection. Two major determinants of clearance vs. persistence have been identified. First, there is general consensus from a number of immunological studies that HCV elimination requires strong and sustained CD4 $^+$  and CD8 $^+$  T cell responses that target multiple epitopes within the different HCV proteins (reviewed in [18]). Of note, HCV is eliminated predominantly by non-cytolytic effector mechanisms, mostly by IFN $\gamma$  driven ISG expression in hepatocytes [15,19]. Second, there is a strong association between the IFN $\lambda$ 4 genotype and spontaneous clearance. Genetic variations of the IFN $\lambda$ 4 gene locus result in a) no IFN $\lambda$ 4 protein, b) production of the wild-type IFN $\lambda$ 4 protein with potent antiviral activity [20] or c) production of a mutant IFN $\lambda$ 4 protein with 10 times lower activity [21]. Paradoxically, the capability to produce the fully active IFN $\lambda$ 4 wild-type does not help the host to eliminate HCV. On the contrary, IFN $\lambda$ 4 wild-type producers have significantly lower spontaneous clearance rates [11,12,21] (see below for details).

The molecular mechanisms that link IFN $\lambda$ 4 genotypes with the cellular immune response in AHC are presently unknown. It has not been shown so far if IFN $\lambda$ 4 is indeed produced in AHC. Furthermore, the immune cell type that could respond to and be (negatively) regulated by IFN $\lambda$ 4 is elusive. Dickensheets and colleagues could not find IFN $\lambda$  induced ISG expression in lymphocytes or monocytes [22], two cell types that are central for regulating anti-HCV immune responses or for exerting antiviral effector mechanisms. More recently, Blazek and colleagues identified neutrophils as IFN $\lambda$  responsive regulators of Th17 and  $\gamma\delta$  T cells in a mouse collagen-induced arthritis model [23]. It remains to be clarified if neutrophils could also be involved in a similar cell-cell network during AHC.

Despite the lack of direct evidence, a central role of IFN $\lambda$ 4 in controlling outcome in AHC is supported by compelling indirect evidence. IFN $\lambda$ 4 comes in two variants that differ in only one amino acid at position 70. The variant with a proline at

this position (IFN $\lambda$ 4 70P) has full antiviral activity, whereas IFN $\lambda$ 4 70S with a serine at position 70 is 10 times less active [21]. This drop in activity has dramatic consequences for the rate of spontaneous HCV clearance: patients with IFN $\lambda$ 4 70S producing genotypes clear HCV more than twice as often compared to IFN $\lambda$ 4 70P producers [21]. This significant association is best explained by a central role of IFN $\lambda$ 4 in regulating the immune response in AHC.

### Host-virus interactions in chronic hepatitis C

The immune response in the chronic phase of hepatitis C is characterized by a largely ineffective cellular response caused by T cell exhaustion and the emergence of viral escape mutations (reviewed in [18]), and by a variable activation of the IFN system in the liver that is also ineffective in clearing the virus (reviewed in [18,24])(Fig. 1). The extent of ISG induction is largely determined by the IFN $\lambda$ 4 genotype [21,25–27]. As in the case of AHC, detection of IFN $\lambda$ 4 protein in liver biopsies of patients with CHC and ISG induction has not been reported. But the central role of IFN $\lambda$ 4 in regulating ISG expression is supported by several lines of evidence. First, IFN $\lambda$ 4 mRNA is detectable in a significant proportion of liver biopsies from patients with CHC [28]. Second, ISG expression is significantly correlated with the expression level of the IFN $\lambda$  receptor in liver biopsies [29]. Third, IFN $\alpha$  cannot be the driver of ISG expression because IFN $\alpha$  signaling is refractory in these patients [29,30]. Refractoriness or non-response to IFN $\alpha$  is most likely caused by USP18, a classical ISG itself and a strong inhibitor of IFN $\alpha$  receptor signaling that is highly expressed in the liver of patients with CHC and high ISG expression [19,31,32]. During the 25 years of (Peg) IFN $\alpha$  based treatments for CHC, the clinical consequence of this refractoriness was non-response to treatment (reviewed in [33]). Because of the strong association of the IFN $\lambda$ 4 genotype with response to treatment, IL28B genotyping (a surrogate marker for IFN $\lambda$ 4 genotyping) has been used in the PegIFN $\alpha$  treatment area to guide treatment decisions. Forth, as in AHC, there is a strong effect of the P70S mutation of IFN $\lambda$ 4 on ISG expression in liver biopsies and response to treatment with pegIFN $\alpha$  and ribavirin (see below) [21].

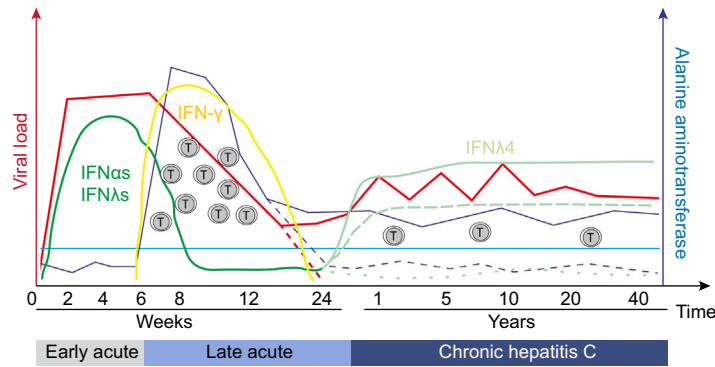
Simultaneous detection of HCV RNA and ISG mRNA by fluorescent *in situ* hybridization has revealed a gradient of ISG expression with HCV infected hepatocytes in the centre [34]. Therefore, infected hepatocytes or IFN producing immune cells in close vicinity are the most likely source of IFN $\lambda$ 4, the supposed driver of ISG expression. Even in small quantities, IFN $\lambda$ 4 binding to IFN $\lambda$  receptors on the same cell or adjacent cells could induce strong ISG induction. This model is supported by the finding that IFN $\lambda$  receptor (IFNLR) expression is inducible

#### Key point

The host immune response to HCV infection has three phases. In the early acute phase, IFN driven innate immunity limits viral replication to some degree, but it is unable to clear the infection. The late acute phase is characterized by a cellular immune response that in a minority of patients leads to spontaneous HCV clearance. In the chronic phase, HCV escapes both innate and adaptive immunity by yet unknown mechanisms. Spontaneous clearance is very rare in chronic hepatitis C.

IFN- $\alpha$  receptor; IFNGR, IFN- $\gamma$  receptor; IFNLR, IFN- $\lambda$  receptor; IRF, interferon regulatory factor; ISG, interferon stimulated gene; KIR, killer cell immunoglobulin-like receptors; MAVS, mitochondrial antiviral signaling protein; MDA5, melanoma differentiation antigen 5; MX1, MX dynamin like GTPase 1; NAFLD, non-alcoholic fatty liver disease; NK cells, natural killer cells; NKT cells, natural killer T cells; pDC, plasmacytoid dendritic cell; PBMC, peripheral blood mononuclear cell; PHH, Primary human hepatocytes; PIAS, protein inhibitor of activated STAT; RAVs, resistance associated variants; RIG-I, retinoic acid inducible gene-I; SNP, single nucleotide polymorphism; SPP1, secreted phosphoprotein 1 (osteopontin); TLR, toll like receptor; TRIF, Toll-IL-1 receptor domain-containing adaptor inducing IFN- $\beta$ ; USP18, Ubiquitin specific peptidase 18; UBP43, ubiquitin specific protease 43 kDa.

## Review



**Fig. 1. Natural course of HCV infection.** Within days after infection, viral load rapidly increases to a plateau of  $10^5$ – $10^7$  IU/ml (red line) (IUs approximately correspond to genome equivalents). In this early phase of acute infection (the first 4–8 weeks), an innate immune response driven by IFN $\alpha$ s and/or IFN $\lambda$ s (green line) might restrict viral replication. With the recruitment of HCV specific T cells in the late phase of AHC, the gene expression profile in the liver switches to an IFN $\gamma$  pattern (yellow line). At the same time, alanine aminotransferase levels increase (blue line) and some patients get icteric. In late AHC, viral replication is strongly inhibited, and in about 30% of patients, HCV is completely eliminated (dashed red line) and alanine transaminase levels return to normal (dashed blue line). In 70%, HCV persists (solid red line), and alanine transaminase remains elevated (solid blue line). In the chronic phase of HCV infection, cellular infiltrates persist at a lower level, but IFN $\gamma$  driven ISG expression disappears. In CHC, the IFN $\lambda$ 4 genotype regulates ISG expression. Patients with the IFN $\lambda$ 4 wild-type genotype have strong ISG expression (light green line), patients with the IFN $\lambda$ 4 P70S variant have intermediate ISG expression (interrupted light green line), and patients without IFN $\lambda$ 4 have no ISG expression (dashed light green line). The light blue line shows the upper limit of normal for alanine transaminase.

by HCV infection [29]. The variable extent of ISG expression in the liver of patients with CHC is the result of a combination of genetically determined IFN $\lambda$ 4 production caused by HCV infection and of the inducible expression of the IFN $\lambda$  receptor that is also caused by HCV infection (Fig. 2). In agreement with this model, inhibition of viral replication by direct acting antiviral therapies induces a rapid downregulation of ISG expression in the liver [35].

IFN $\lambda$ 4 driven ISG expression in the liver has limited antiviral effects. The association of *IL28B* TT genotype (a surrogate for the IFN $\lambda$ 4  $\Delta$ G/ $\Delta$ G “producer” genotype) with lower HCV viral loads [7] suggests that IFN $\lambda$ 4 restricts HCV replication to some extent. However, HCV obviously must have effective escape mechanisms that allow it to persist for decades despite the expression of hundreds of ISGs [30,36,37]. At present, these IFN escape mechanisms have not been identified (reviewed in [18]).

Little is known about host genetics and the cellular immune response to HCV. A hallmark of chronic HCV infection is the presence of functionally impaired virus-specific CD8 $^+$  T cells that are characterized by their inability to secrete antiviral cytokines such as IFN $\gamma$  or to proliferate (reviewed in [38,39]). There is evidence that the impaired cellular immune response is not fixed, but seems to be an active evasion strategy by the virus, and that HCV specific CD8 $^+$  T cell function is restored after successful HCV eradication by direct antiviral IFN-free therapies [40,41].

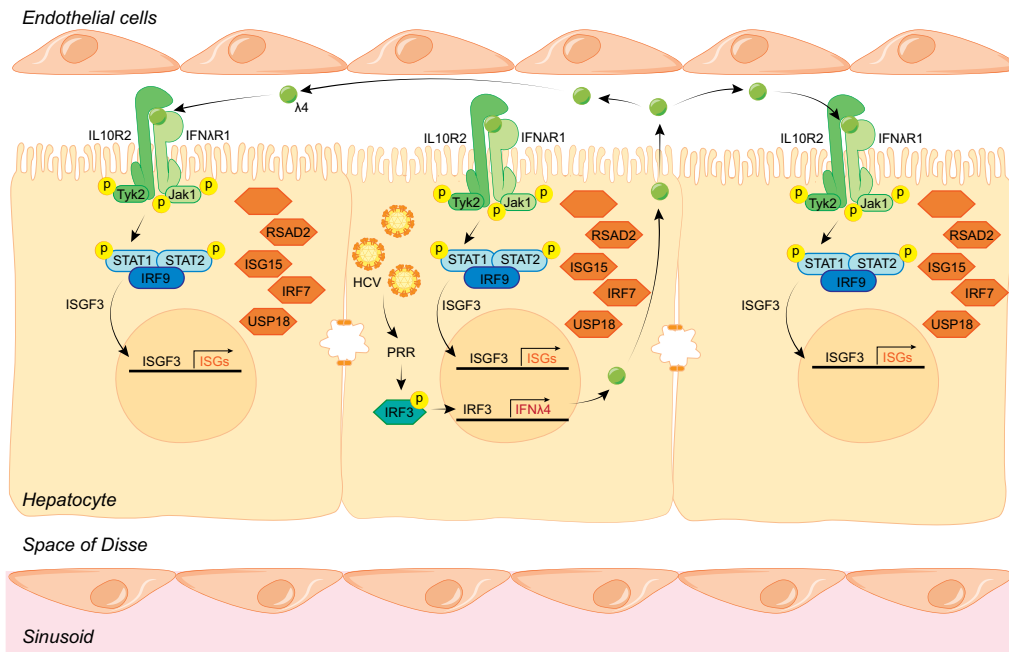
### Genetic associations with spontaneous and treatment-induced HCV clearance

Hepatitis C infection is characterized by a remarkable variability in patients' ability to eliminate the virus, either spontaneously or after treatment [2,3]. In the late 1990's, investigators started to hypothesize that this variability may be due, at least in part, to polymorphisms in the host immune system. Initial studies focused on genes known or supposed to exert an important role on host responses to the pathogen (candidate gene studies) [42,43]. Subsequently, together with the progress in genotyping techniques, another series of studies explored whether polymorphisms within the whole genome influenced HCV clearance (genomewide association studies, GWAS) [44].

Human leukocyte antigen (HLA) genes represent a highly polymorphic family of genes located in the short arm of chromosome 6. They encode molecules that can form stable complexes with foreign peptides and are essential for the presentation of viral antigens to T cells [45,46]. The great diversity of HLA alleles ensures the ability of the immune system to respond to a vast array of pathogenic agents. Numerous studies have associated HLA alleles with susceptibility to infectious diseases. Along this line, several studies have explored whether HLA alleles are associated with spontaneous HCV clearance (reviewed in [42–44]) and, to a lesser extent, response to IFN-based treatment. These studies were often performed in a small number of patients and in different ethnic populations, and used different methods for HLA typing as well as different clinical phenotypes, making it difficult to compare results one study to another [42,43]. Despite these limitations, some associations appear to be relatively consistent, and/or have been validated in GWAS, including those of spontaneous HCV clearance with HLA-DQB1\*03 and HLA-DRB1\*11 [42–44,47–50].

During the early 2000s, accumulating evidence suggested that NK cells have an important role in the immune response against HCV, during both acute and chronic infection [51]. This antiviral function is mediated by killer cell immunoglobulin-like receptors (KIR) that are expressed on NK cells and recognize conserved epitopes on HLA class I molecules. KIR can exert either an activator or an inhibitor effect on NK cell function. They are encoded by a family of highly polymorphic genes located within the leukocyte receptor complex in chromosome 19. Several investigators analyzed whether the carriage of specific KIR and/or KIR ligands are associated with the outcome of HCV infection. In a study of 1,037 patients, spontaneous HCV clearance was associated with the presence of KIR2DLR3 with homozygosity for the HLA-C1 ligand [52]. Although the association of spontaneous HCV clearance with KIR2DLR3 and HLA-C1 homozygosity was [53] or tended to be [54] replicated, it was not confirmed by others

\* Corresponding authors.  
Addresses: Department of Biomedicine, University of Basel, Zentrum für Lehre und Forschung, Hebelstrasse 20, CH-4031 Basel, Switzerland. Tel.: +41 61 265 33 62; fax: +41 61 265 38 47 (M.H. Heim), or Service des Maladies Infectieuses, BH10 – 537, Rue du Bugnon 46, CH-1011 Lausanne, Switzerland. Tel.: +41 (0)21 314 43 79; fax: +41 (0)21 314 05 97 (P.-Y. Bochud), or Storr Liver Centre, Westmead Institute for Medical Research, Westmead Hospital and University of Sydney, Westmead, NSW, Australia 2145. Tel.: +61 (0)2 98457705; fax: +61 (0)2 96357582 (J. George).  
E-mail addresses: Markus.heim@unibas.ch (M.H. Heim), Pierre-Yves.Bochud@chuv.ch (P.-Y. Bochud), jacob.george@sydney.edu.au (J. George).



**Fig. 2. The central role of IFN $\lambda$ 4 in ISG induction in CHC.** Sensing of HCV pathogen associated molecular patterns (PAMPs) and consecutive activation of signaling pathways induces the phosphorylation and activation of IRF3 and NF $\kappa$ B (not shown). IFN $\lambda$ 4 (dark green) is produced in patients with the IFN $\lambda$ 4  $\Delta$ G/ $\Delta$ G genotype. IFN $\lambda$ 4 is secreted and binds to IFN $\lambda$  receptors on the same cell or on neighboring cells (autocrine and paracrine action). IL10R2 is constitutively expressed, whereas IFN $\lambda$ AR1 is inducible expressed in hepatocytes in CHC by yet unknown mechanisms. Signaling through the IFN $\lambda$  receptor activates the Jak-STAT pathway and results in the expression of hundreds of ISGs (orange).

[55–57]. Reversely, carriage of KIR2DS3 together HLA-C2 was consistently associated with failure to clear HCV, either spontaneously [54] or after treatment in a cohort of HIV-1/HCV co-infected patients [58].

In addition to studies focusing on HLA and KIR genes polymorphisms, >100 studies analyzed the association of ~200 polymorphisms from ~80 other host genes with spontaneous or treatment-induced HCV clearance. The relevance of these associations is often difficult to establish, as studies are heterogeneous in terms of size and quality, report data from different ethnic groups, include patients infected with different HCV genotypes and/or different HIV serostatus. Many polymorphisms have been tested only in a single study or, when tested in  $\geq 2$ , their associations with the clinical phenotype(s) are often not replicated. However, some associations have been replicated. Two SNPs in *SPP1*, the gene encoding osteopontin, a protein exerting cytokine-like functions that are important for efficient Th-1 immune responses, were consistently associated with increased (ins155G) and decreased (rs11730582) sustained viral response (SVR) rates after treatment with IFN-based treatments [59–61]. Another SNP (rs17000900) located within *MX1*, a gene belonging to the group of ISGs, was associated with increased SVR rates [60,62], although this association was not

observed by others [59]. There is a clear need for well conducted systematic reviews and meta-analyses to reconcile the results from candidate genes studies.

The most relevant association of host genes with hepatitis C-related phenotypes resulted from 4 GWAS [7–10]. These studies identified two SNPs located in the q13.12/13 regions of chromosome 19, within the *IFNλ3* (formerly *IL28B*) locus, (rs12979860 and rs8099917), which were associated with response to PegIFN [7–10] and ribavirin therapy as well as and spontaneous HCV clearance [8]. The association was replicated in hundreds of studies including >25,000 patients from different ethnic groups. It is among the most consistent ever described to influence the course of an infectious disease in the general population. However, the mechanisms by which these polymorphisms influenced host immune responses during acute and chronic HCV infection have not been understood for several years. Of note, GWAS did not detect the associations of HLA and KIR genes with spontaneous and/or treatment-induced clearance, at least not at the GWAS significance level. This might be due to several factors, including a suboptimal coverage of these highly polymorphic regions in the GWAS, difficulties encountered at the time in trying reconciling KIR/HLA genotypes with SNPs data and the fact that GWAS and HLA/KIR studies did often not target



## Review

### Key point

In the current era of IFN-free direct acting antiviral (DAA) combination therapies with cure rates exceeding 90%, the role of all genetic predictors involving *IFNλ* genotype has diminished.

### Key point

*IL28B* corresponds to interferon lambda 3 (*IFNλ3*). One of the single nucleotide polymorphisms (SNPs) in the locus, rs368234815 ΔG, has a functional consequence: It determines the expression of the more recently discovered *IFNλ4*.

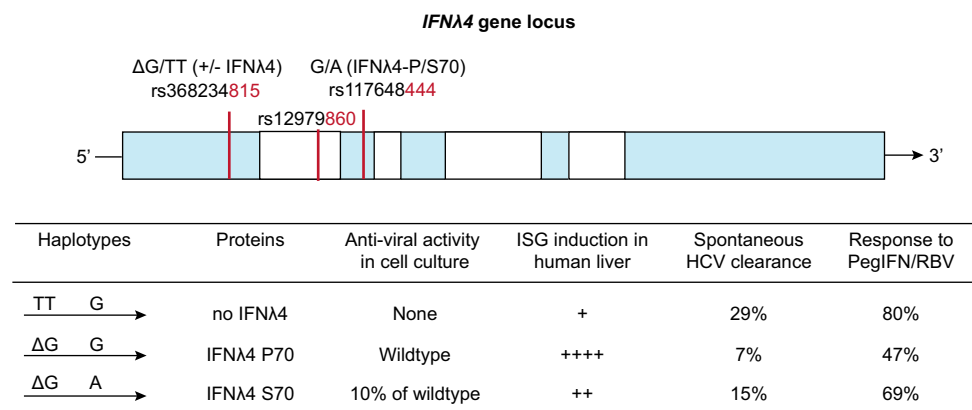
the same populations (most HLA studies were conducted among patients with spontaneous clearance, while most GWAS were conducted among treatment responders and non-responders).

By using a combination of gene mapping and functional experiments, two groups of investigators identified a novel polymorphism within the *IFNλ* locus (rs368234815, ΔG). This appeared as a better predictor of viral clearance than previously identified SNPs [11,12] and resulted in a frameshift mutation leading to the expression of *IFNλ4*. The *IFNλ4* gene was subsequently shown to contain an additional SNP leading to an amino acid substitution (P70S, rs117648444) which alters the activity of the *IFNλ4* protein and is associated with an improved HCV clearance [21]. Altogether, the variability within the *IFNλ*s seems to result from these two polymorphisms (rs368234815 and rs117648444) that determine three haplotypes, each associated with a different pattern of *IFNλ4* expression (Fig. 3) [21]. The TT G haplotype is predicted not to produce *IFNλ4*, the ΔG G is predicted to express the *IFNλ4*-P70 variant and the ΔG A is predicted to express the *IFNλ4*-S70 variant. Based on their haplotypic combinations (diplotypes), individuals can be stratified into 3 different groups. Patients who do not produce *IFNλ4* have low ISG expression in the liver and a good ability to clear HCV either spontaneously or after IFN-based treatment. Those who produce the functional P70 form of *IFNλ4* produce important amounts of ISG in the liver and have a low ability to clear HCV. Those who produce the inactive S70 form of *IFNλ4* and have an intermediate ability to clear HCV (Fig. 3).

A GWAS demonstrated that an inosine triphosphate pyrophosphatase (ITPA) genetic variant modulates thrombocytopenia, and RBV-induced anemia in HCV genotype 1 [63–65].

In the current era of IFN-free direct acting antiviral (DAA) combination therapies with cure rates exceeding 90%, the role of all predictors involving *IFNλ* genotype has diminished. Having said this, a role for *IFNλ* genotype in response to DAA combinations with simeprevir/PegIFN/RBV, sofosbuvir/PegIFN/RBV and faldaprevir/PegIFN/RBV has been reported [66–68]. Even with some IFN-free regimens, a role for *IFNλ* genotype has been demonstrated, such as with aldaprevir, deleobuvir and RBV, and simeprevir and sofosbuvir without RBV [69]. Further, it has been reported that *IFNλ* genotype affects viral kinetics for some IFN-free regimens, as for example, in patients treated with sofosbuvir/RBV [70], and those receiving a combination of mericitabine and danoprevir [71].

More recently, the association of genetic variants of the *IFNλ4* (*IL28B* genotype) with HCV mutations that are associated with drug resistance (resistance associated variants, RAVs) has been investigated. The authors found that naturally occurring RAVs against NS3/4A protease inhibitors and non-nucleoside NS5B polymerase inhibitors were not significantly associated with the *IFNλ4* genotype. However, the NS5A RAV Y93H was significantly associated. Interestingly, patients with the genotype that is beneficial for PegIFNα/RBV treatments were found to have significantly higher rates of the Y93H mutation in the NS5A gene of HCV already before treatment with DAAs [72]. Along the same line, increasing evidence showed that viral genetic variation can be induced by continuous selection by host immune pressure [73,74], suggesting that subsequent viral escape can be determined by the combination of specific viral genotypes together with a specific host immune pattern [75]. It remains to be clarified if this finding has clinical relevance, for example



**Fig. 3. Genetic polymorphisms in the *IFNλ4* gene determine IFN stimulated gene induction and viral clearance.** The rs368234815 and rs117648444 polymorphisms determine 3 haplotypes that predict a different expression of *IFNλ4* (none, 70P variant and 70S variant). Subsequent haplotypic combinations (diplotypes) determine the ability of patients to produce *IFNλ4*. Patients who do not produce *IFNλ4*, those who produce its active 70P form and those who produce its inactive 70S form have different patterns of liver ISG expression and a different ability to clear HCV [21].

for the choice of specific DAA regimens for individual patients.

In total, these data suggest that the pattern of innate immunity regulated by *IFNλ* genotype contributes to the outcome of HCV treatment, even without IFN therapy with certain regimens.

### Host-viral interactions and liver disease progression: evidence for a role for genetic variation

Though the relevance of host genetic variation to the outcome of DAA treatment is less obvious, recent reports highlight their role to other liver disease phenotypes during the course of viral hepatitis, including to liver inflammation, steatosis, fibrosis and HCC.

Virus-induced host responses leading to hepatic inflammation forms the pathogenic basis for fibrosis progression leading to cirrhosis, and eventually to HCC. While factors including age, duration of infection, gender, insulin resistance and steatosis [76,77] are associated with disease progression in chronic viral hepatitis, these known risk factors explain only approximately a third of the variability in progression [76]. This implies a possible role for genetic variation, and more importantly, their interaction together (Gene x Gene) and with environmental factors (Gene x Environment). This is supported by results from a recent prospective cohort study of 60 twin pairs suggesting that the heritability of fibrosis was of the order of 50% [78].

#### Genetic variation in interferon lambda and other liver disease phenotypes

As discussed earlier, the host immune response to hepatitis C viruses is crucial to the outcomes of infection. Thus, it could be expected that genetic variation in this response can modulate the extent of hepatic inflammation, and through this, the progression to fibrosis. Consistent with this notion, there is now robust evidence that the favorable “responder” *IFNλ* genotype is associated with higher hepatic inflammatory activity and an acceleration of fibrosis progression in CHC [79,80]. It is noteworthy, that multiple reports have shown that this effect is more predominant in subjects infected with HCV genotype 3 [79–81] and that this genotype is associated with higher fibrosis progression rates [82] and a two fold increase in HCC and mortality risk compared to other HCV genotypes [83]. Several other reports suggest that HCV-1 is associated with greater spontaneous clearance compared to HCV non-1 [84] and that those with rs12979860 CC are more likely to be chronically infected with HCV-3 than with HCV non-3 [80,85]. Whether host immune responses and *IFNλ* genotype could be

involved in linking together these effects is unknown. Notably, consistent with the effect of *IFNλ* genotype on the acceleration of liver injury, an association has also been observed with long-term clinical outcomes. In one study of 400 untreated patients from the HALT-C trial, patients with a responder *IFNλ* genotype were twice as likely to develop adverse clinical outcomes, compared to subjects with the non-responder genotype during 3.8 years follow-up [86]. It is noteworthy however that another smaller Italian study of 264 compensated HCV-cirrhotic patients but with longer follow-up duration (median duration of follow up 14.8 years) failed to demonstrate any association with clinical outcomes [87]. Collectively, these data on balance imply an important role for genetic variation in *IFNλ* to disease progression and clinical outcomes.

Data on the association of *IFNλ* genotypes to the risk of HCC occurrence and recurrence after therapy are inconclusive. However, the overall available evidence suggests an opposite association for *IFNλ* genotypes to the risk of HCC. The reports suggest that the risk of HCC may be higher in subjects with the non-responder *IFNλ* genotypes [88–90]. Similar observations were noticed in the context of HCV-related liver transplantation [91]. These studies should however be interpreted with caution given the potential risk for selection bias as more subjects with responder genotypes would have cleared HCV on IFN-based therapy. The net effect would be enrichment of the group of HCC with those carrying the non-response *IFNλ* genotype [92].

#### Other genetic variation and virus-related fibrosis and cirrhosis

Two recent GWAS have investigated genetic variants associated with the risk of HCV-related liver fibrosis and cirrhosis. The first, in a cohort of 2,342 biopsy-proven patients of European descent (1161 in a discovery cohort and 219 in a replication cohort) [93] revealed polymorphisms in apoptosis-related genes such as rs4374383, rs9380516 and rs16851720 located within or close to *MERTK*, *TULP1* and *RNF7* genes respectively, to be associated with fibrosis progression. The second GWAS compared 682 Japanese patients with HCV-associated liver cirrhosis to 1045 CHC patients without cirrhosis. This revealed two SNPs (rs910049 and rs3135363) located within the major histocompatibility complex (*MHC*) region on chromosome 6p21 to be significantly associated with the progression from CHC to cirrhosis [94]. In this GWAS, liver disease staging for most subjects was not biopsy-based. The replication and translational impact of the findings however, are still limited and needs validation, though they undoubtedly provide clues to pathogenesis.

## Review

### Genetic variation and virus-associated HCC

Two GWAS on HCV-related HCC have been reported [95,96]. These two studies were conducted in Japanese patients using the same chip (Illumina HumanHap610-Quad Genotyping Bead Chip), while they identified different risk variants. The first study [95] performed in 721 individuals with HCV-induced HCC and 2,890 HCV negative controls, identified a SNP (rs2596542) located in the 5'flanking region of the MHC class I-related chain A (*MICA*) on 6p21.33 to be strongly linked with HCV-related HCC. The findings were replicated in 673 cases and 2,596 controls, and with further analyses in 1730 individuals with CHC with no cirrhosis or HCC. A potential limitation is the fact that the control cohort was not cirrhotic, while virtually all HCV-related HCC occurs in the context of cirrhosis. Furthermore, the use of HCV negative subjects as the control group could be considered inappropriate as HCV infection *per se* is associated with fibrosis progression and therefore ultimately, an increased risk of HCC compared to healthy controls. Thus, the identified variants might be associated with progression to cirrhosis rather than HCC. The second GWAS compared 212 Japanese individuals with HCV-related HCC to 765 individuals with CHC without HCC and a replication cohort of 710 cases and 1625 controls. This study identified an intronic SNP (rs1012068) located in the *DEPDC5* locus on chromosome 22q12.2–3, as significantly associated with susceptibility to HCC [96]. Again, biopsy data was not available and a low platelet count ( $<10 \times 10^4/\mu\text{l}$ ) was used as a surrogate marker for cirrhosis.

### Other genes and liver phenotypes in chronic hepatitis C

A landmark GWAS in 2009 revealed that a variant (rs738409 encoding for the I148M substitution) in the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) was the strongest genetic variant associated with non-alcoholic fatty liver disease (NAFLD) [97]. This variant influences liver fat without affecting insulin resistance or body composition and has been consistently associated with the full spectrum of histological features in NAFLD [98,99]. More recently, the role of virus-host interactions in relation to the *PNPLA3* polymorphism has been explored. This indicated an association with hepatic steatosis, especially in patients infected with HCV genotype non-3, and as well to fibrosis progression [100,101].

Another non-synonymous genetic variant within the transmembrane 6 superfamily member 2 (*TM6SF2*) gene (rs58542926 c.449 C>T, p.E167K) has been shown by an exome wide association study to increase hepatic triglyceride content in NAFLD [102]. Other studies have linked the same variant to fibrosis progression and the risk of HCC

in NAFLD [103]. With regard to a role for this variant in modulating liver histology in viral liver disease, it has been reported to be associated with steatosis in CHC, while any effect on fibrosis was modest in CHC [101,104].

### miRNAs and virus-host interactions

Accumulating evidence suggests a role for human miRNAs in modulating HCV viral infectivity, cell tropism and host immune responses [105]. This interaction appears to be dual; cellular miRNAs directly target defined viral genomes or transcripts while they are also able to indirectly target viruses through miRNA-mediated regulation of specific host factors. The outcome of this miRNA-virus-host interplay can have either negative (antiviral) or positive (proviral) consequence for the virus. Furthermore, this interaction could be virus-dependent [106]. miR-122, accounts for 70% and 52% of the whole hepatic miRNome in adult mouse and human, respectively, and represents the best described example so far [107]. miR-122 has proviral effects on HCV viral translation, replication and infectious particle production and thus it represents a target for antiviral therapy [108,109]. Interestingly, in contrast to its proviral role in HCV infection, miR-122 appears to restrict HBV replication, though the mechanisms are unclear. Consistent with the antiviral effect of miR-122 on HCV replication, administration of Miravirsin, a miR-122 inhibitor results in a dose dependent and prolonged decrease in HCV RNA levels in CHC patients [110].

### Genes and host-virus interactions: open questions and challenges

Undoubtedly, the outcome of HCV infection including liver disease progression is modulated by the host genetic background and as well, (Gene x Gene) and (Gene x Environment) interactions. However, a major challenge remains that the known loci of all complex phenotypes tend to explain only a tiny proportion of the heritability of the phenotypes [111]. The same is true even for complex diseases with high heritability such as age-related macular degeneration [112,113]. A recent study by us indicates that this is also the case for liver fibrosis. Thus, a significant portion of the fibrosis progression rate could not be explained by common cofactors including clinical variables and SNPs discovered by GWAS, based on a low pseudo- $R^2$  of the multivariate logistic regression model of fibrosis progression [114]. This observation indicates that there are additional loci affecting liver injury that remain to be uncovered even after the GWAS era. It should be noted that GWAS by their design, use conservative significance thresholds in order to guard against false positive signals (i.e., adjusted threshold of  $10^{-6}$  to  $10^{-8}$  for most of the current available SNP chips). This can

### Key point

Genetic variations at the *IFN $\lambda$*  locus, but also at other loci, modulate clinically important liver phenotypes, including inflammation, fibrosis progression and the development of hepatocellular cancer.

lead to loss of real and important associations, prudent if they are of modest effect or a low minor allele frequency. Further, there is expected, but as yet an undetermined contribution from rare variants to complex phenotypes [115].

Currently, there are concerns about the clinical utility of genetic information for most complex phenotypes [116,117], since virtually all the known risk variants have low clinical classifying performance. However, it is possible that as our understanding of the genetic architecture of fibrosis and host-virus interactions improves, more genes that independently associate with disease are identified (even if of modest effect) and the predictive value of novel cumulative polygenic scores with or without clinical variables will improve. In this context, an earlier study [118] constructed a gene-based cirrhosis risk score (CRS) consisting of 7 variants identified by a genome scan that selected 361 markers in a derivation cohort (N = 420), and then validated the 7 gene signature in a second cohort of 154 patients. Further, a recent international multi-centre study utilized systematic un-biased non-parametric, machine learning methods designed for predictive modeling, and incorporated *IFN $\lambda$*  genotype with other simple clinical variables in a novel non-invasive decision tree model (Fibro-GENE) for prediction of liver fibrosis risk in a cohort of >4000 patients [119].

Another major challenge to understanding host-virus interactions is filling the gaps from GWAS discovery to determine the functional basis for their effects on phenotype. The same extends to a potential role for non-sequence based genomic variation. This includes a focus on the role of epigenetic changes that can influence gene expression and cell phenotype through a variety of regulatory processes including DNA methylation, histone modification, transcriptional control, chromatin remodeling and non-coding RNAs [120]. The era of the epigenome-wide association study (EWAS) of large numbers of samples has begun in other complex phenotypes such as body mass index, metabolic traits, rheumatoid arthritis and multiple sclerosis [121]. Based on the experience with GWAS, it is inevitable that EWAS studies will extend to liver diseases. However, unlike genetics, conducting meaningful EWAS will be challenging as there are a range of potentially vital confounding factors that need to be considered, such as age, sex, drug exposure and multiple environmental factors.

Another concern is that epigenetic signatures are highly plastic and with tissue-specific variation. A final issue is the ability to discriminate cause from effect. This is crucial as unlike genetic modifiers of disease, unhealthy epigenetic modifications could result in aberrant expression of various genes without directly introducing changes to the DNA sequence [122]. Lastly, other types of genetic variation such as copy number variants (CNVs) including both duplications (copy number gains) and deletions (copy number losses) of genetic material, undoubtedly modulate host-viral interactions. Indeed, CNVs have been estimated to affect ~12% of the human genome [123].

## Conclusions

While much has been understood through recent scientific advancements about the role of genes to host-virus interactions, much still remains to be fathomed with regard to the role of genes to complex liver phenotypes. The challenge of curing hepatitis C to prevent adverse liver-related outcomes has nearly been won. The lessons learnt along this journey in relation to host-virus interactions will no doubt enable us to more effectively understand and treat the rising burden of metabolic liver disease.

## Financial support

The work was supported by Swiss National Science Foundation grant 320030\_130243 and 310030\_166202 to MHH. PYB is supported by the Swiss National Foundation (32003B-127613 and 324730-144054), the Leenaards Foundation and, the Santos-Suarez Foundation. JG is supported by the Robert W. Storr Bequest to the Sydney Medical Foundation, University of Sydney; a National Health and Medical Research Council of Australia (NHMRC) Program Grant (1053206) and Project grants (APP1107178 and APP1108422).

## Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

## References

Author names in bold designate shared co-first authorship.

- [1] Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005;5:558–567.
- [2] Santantonio T, Wiegand J, Gerlach JT. Acute hepatitis C: current status and remaining challenges. *J Hepatol* 2008;49:625–633.
- [3] Lauer GM, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001;345:41–52.
- [4] Freeman AJ, Dore GJ, Law MG, Thorpe M, Von Overbeck J, Lloyd AR, et al. Estimating progression to cirrhosis in chronic hepatitis C virus infection. *Hepatology* 2001;34:809–816.
- [5] Thein HH, Yi Q, Dore GJ, Krahn MD. Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. *Hepatology* 2008;48:418–431.



## Review

- [6] El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012;142:1264–1273, e1.
- [7] 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.
- [8] 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, 45, e1–e7.
- [9] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009;41:1105–1109.
- [10] Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009;41:1100–1104.
- [11] 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.
- [12] Bibert S, Roger T, Calandra T, Bochud M, Cerny A, Semmo N, et al. IL28B expression depends on a novel TT/-G polymorphism which improves HCV clearance prediction. *J Exp Med* 2013;210:1109–1116.
- [13] Wack A, Terczynska-Dyla E, Hartmann R. Guarding the frontiers: the biology of type III interferons. *Nat Immunol* 2015;16:802–809.
- [14] Bigger CB, Brasky KM, Lanford RE. DNA microarray analysis of chimpanzee liver during acute resolving hepatitis C virus infection. *J Virol* 2001;75:7059–7066.
- [15] Thimme R, Bukh J, Spangenberg HC, Wieland S, Pemberton J, Steiger C, et al. Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. *Proc Natl Acad Sci U S A* 2002;99:15661–15668.
- [16] Major ME, Dahari H, Mihalik K, Puig M, Rice CM, Neumann AU, et al. Hepatitis C virus kinetics and host responses associated with disease and outcome of infection in chimpanzees. *Hepatology* 2004;39:1709–1720.
- [17] Dahari H, Major M, Zhang X, Mihalik K, Rice CM, Perelson AS, et al. Mathematical modeling of primary hepatitis C infection: noncytolytic clearance and early blockage of virion production. *Gastroenterology* 2005;128:1056–1066.
- [18] Heim MH, Thimme R. Innate and adaptive immune responses in HCV infections. *J Hepatol* 2014;61:S14–S25.
- [19] Dill MT, Makowska Z, Duong FH, Merkofer F, Filipowicz M, Baumert TF, et al. Interferon-gamma-Stimulated Genes, but Not USP18, are expressed in livers of patients with acute hepatitis C. *Gastroenterology* 2012;143:777–786, e6–e6.
- [20] Hamming OJ, Terczynska-Dyla E, Vieyres G, Dijkman R, Jorgensen SE, Akhtar H, et al. Interferon lambda 4 signals via the IFNlambda receptor to regulate antiviral activity against HCV and coronaviruses. *EMBO J* 2013;32:3055–3065.
- [21] Terczynska-Dyla E, Bibert S, Duong FH, Krol I, Jorgensen S, Collinet E, et al. Reduced IFNlambda4 activity is associated with improved HCV clearance and reduced expression of interferon-stimulated genes. *Nat Commun* 2014;5:5699.
- [22] Dickensheets H, Sheikh F, Park O, Gao B, Donnelly RP. Interferon-lambda (IFN-lambda) induces signal transduction and gene expression in human hepatocytes, but not in lymphocytes or monocytes. *J Leukoc Biol* 2013;93:377–385.
- [23] Blazek K, Eames HL, Weiss M, Byrne AJ, Perocheau D, Pease JE, et al. IFN-lambda resolves inflammation via suppression of neutrophil infiltration and IL-1beta production. *J Exp Med* 2015;212:845–853.
- [24] Heim MH. Innate immunity and HCV. *J Hepatol* 2013;58:564–574.
- [25] Urban TJ, Thompson AJ, Bradrick SS, Fellay J, Schuppan D, Cronin KD, et al. IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated genes in patients with chronic hepatitis C. *Hepatology* 2010;52:1888–1896.
- [26] 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.
- [27] 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.
- [28] Amanzada A, Kopp W, Spengler U, Ramadori G, Mihm S. Interferon-lambda4 (IFNL4) Transcript Expression in Human Liver Tissue Samples. *PLoS One* 2013;8:e84026.
- [29] Duong FH, Trincucci G, Boldanova T, Calabrese D, Campana B, Krol I, et al. IFN-lambda receptor 1 expression is induced in chronic hepatitis C and correlates with the IFN-lambda3 genotype and with nonresponsiveness to IFN-alpha therapies. *J Exp Med* 2014;211:857–868.
- [30] 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 U S A* 2008;105:7034–7039.
- [31] Makowska Z, Duong FH, Trincucci G, Tough DF, Heim MH. Interferon-beta and interferon-lambda signaling is not affected by interferon-induced refractoriness to interferon-alpha in vivo. *Hepatology* 2011;53:1154–1163.
- [32] Sarasin-Filipowicz M, Wang X, Yan M, Duong FH, Poli V, Hilton DJ, et al. Alpha interferon induces long-lasting refractoriness of JAK-STAT signaling in the mouse liver through induction of USP18/UBP43. *Mol Cell Biol* 2009;29:4841–4851.
- [33] Heim MH. 25 years of interferon-based treatment of chronic hepatitis C: an epoch coming to an end. *Nat Rev Immunol* 2013;13:535–542.
- [34] Wieland S, Makowska Z, Campana B, Calabrese D, Dill MT, Chung J, et al. Simultaneous detection of hepatitis C virus and interferon stimulated gene expression in infected human liver. *Hepatology* 2014;59:2121–2130.
- [35] Lanford RE, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science* 2010;327:198–201.
- [36] Chen L, Borozan I, Feld J, Sun J, Tannis LL, Coltescu C, et al. Hepatic gene expression discriminates responders and nonresponders in treatment of chronic hepatitis C viral infection. *Gastroenterology* 2005;128:1437–1444.
- [37] Asselah T, Bieche I, Narguet S, Sabbagh A, Laurendeau I, Ripault MP, et al. Liver gene expression signature to predict response to pegylated interferon plus ribavirin combination therapy in patients with chronic hepatitis C. *Gut* 2008;57:516–524.
- [38] Klennerman P, Thimme R. T cell responses in hepatitis C: the good, the bad and the unconventional. *Gut* 2012;61:1226–1234.
- [39] Rehhermann B. Hepatitis C virus versus innate and adaptive immune responses: a tale of coevolution and coexistence. *J Clin Invest* 2009;119:1745–1754.
- [40] Martin B, Hennecke N, Lohmann V, Kayser A, Neumann-Haefelin C, Kukulj G, et al. Restoration of HCV-specific CD8+ T cell function by interferon-free therapy. *J Hepatol* 2014;61:538–543.
- [41] Burchill MA, Golden-Mason L, Wind-Rotolo M, Rosen HR. Memory re-differentiation and reduced lymphocyte activation in chronic HCV-infected patients receiving direct-acting antivirals. *J Viral Hepat* 2015;22:983–991.
- [42] Kuniholm MH, Kovacs A, Gao X, Xue X, Marti D, Thio CL, et al. Specific human leukocyte antigen class I and II alleles associated with hepatitis C virus viremia. *Hepatology* 2010;51:1514–1522.
- [43] Singh R, Kaul R, Kaul A, Khan K. A comparative review of HLA associations with hepatitis B and C viral infections across global populations. *World J Gastroenterol* 2007;13:1770–1787.
- [44] Matsuura K, Tanaka Y. Host genetic variants influencing the clinical course of hepatitis C virus infection. *J Med Virol* 2016;88:185–195.
- [45] McKiernan SK. D. Immunogenetics of hepatitis C virus. *J Viral Hepat* 2000;7:13–14.
- [46] Alric L, Izopet J, Fort M, Vinel JP, Fontenelle P, Orfila C, et al. Study of the association between major histocompatibility complex class II genes and the response to interferon alpha in patients with chronic hepatitis C infection. *Hum Immunol* 1999;60:516–523.
- [47] Alric L, Fort M, Izopet J, Vinel JP, Charlet JP, Selves J, et al. Genes of the major histocompatibility complex class II influence the outcome of hepatitis C virus infection. *Gastroenterology* 1997;113:1675–1681.
- [48] Alric L, Fort M, Izopet J, Vinel JP, Bureau C, Sandre K, et al. Study of host- and virus-related factors associated with spontaneous hepatitis C virus clearance. *Tissue Antigens* 2000;56:154–158.
- [49] Duggal P, Thio CL, Wojcik GL, Goedert JJ, Mangia A, Latanich R, et al. Genome-wide association study of spontaneous resolution of hepatitis C virus infection: data from multiple cohorts. *Ann Intern Med* 2013;158:235–245.
- [50] Miki D, Ochi H, Takahashi A, Hayes CN, Urabe Y, Abe H, et al. HLA-DQB1\*03 confers susceptibility to chronic hepatitis C in Japanese: a genome-wide association study. *PLoS One* 2013;8:e84226.
- [51] Gardiner CM. NK cell function and receptor diversity in the context of HCV infection. *Front Microbiol* 2015;6:1061.
- [52] Khakoo SI, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* 2004;305:872–874.
- [53] Knapp S, Warshow U, Hegazy D, Brackenbury L, Guha IN, Fowell A, et al. Consistent beneficial effects of killer cell immunoglobulin-like receptor 2DL3 and group 1 human leukocyte antigen-C following exposure to hepatitis C virus. *Hepatology* 2010;51:1168–1175.

- [54] Dring MM, Morrison MH, McSharry BP, Guinan KJ, Hagan R, Irish HCVRC, et al. Innate immune genes synergize to predict increased risk of chronic disease in hepatitis C virus infection. *Proc Natl Acad Sci U S A* 2011;108:5736–5741.
- [55] Rauch A, Laird R, McKinnon E, Telenti A, Furrer H, Weber R, et al. Influence of inhibitory killer immunoglobulin-like receptors and their HLA-C ligands on resolving hepatitis C virus infection. *Tissue Antigens* 2007;69:237–240.
- [56] Montes-Cano MA, Caro-Oleas JL, Romero-Gomez M, Diago M, Andrade R, Carmona I, et al. HLA-C and KIR genes in hepatitis C virus infection. *Hum Immunol* 2005;66:1106–1109.
- [57] Thoens C, Berger C, Trippler M, Siemann H, Lutterbeck M, Broering R, et al. KIR2DL3(+)NKG2A(-) natural killer cells are associated with protection from productive hepatitis C virus infection in people who inject drugs. *J Hepatol* 2014;61:475–481.
- [58] Keane C, O'Shea D, Reiberger T, Peck-Radosavljevic M, Farrell G, Bergin C, et al. Variation in both IL28B and KIR2DS3 genes influence pegylated interferon and ribavirin hepatitis C treatment outcome in HIV-1 co-infection. *PLoS One* 2013;8:e66831.
- [59] Naito M, Matsui A, Inao M, Nagoshi S, Nagano M, Ito N, et al. SNPs in the promoter region of the osteopontin gene as a marker predicting the efficacy of interferon-based therapies in patients with chronic hepatitis C. *J Gastroenterol* 2005;40:381–388.
- [60] Angelo AL, Cavalcante LN, Abe-Sandes K, Machado TB, Lemaire DC, Malta F, et al. Myxovirus resistance, osteopontin and suppressor of cytokine signaling 3 polymorphisms predict hepatitis C virus therapy response in an admixed patient population: comparison with IL28B. *Clinics (Sao Paulo)* 2013;68:1325–1332.
- [61] Shaker O, El-Shehaby A, Fayed S, Zahra A, Marzouk S, El Raziky M. Osteopontin gene polymorphisms as predictors for the efficacy of interferon therapy in chronic hepatitis C Egyptian patients with genotype 4. *Cell Biochem Funct* 2013;31:620–625.
- [62] Hijikata M, Mishihiro S, Miyamoto C, Furuichi Y, Hashimoto M, Ohta Y. Genetic polymorphism of the MxA gene promoter and interferon responsiveness of hepatitis C patients: revisited by analyzing two SNP sites (-123 and -88) in vivo and in vitro. *Intervirology* 2001;44:379–382.
- [63] Fellay J, Thompson AJ, Ge D, Gumbs CE, Urban TJ, Shianna KV, et al. ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. *Nature* 2010;464:405–408.
- [64] Tanaka Y, Kurosaki M, Nishida N, Sugiyama M, Matsuura K, Sakamoto N, et al. Genome-wide association study identified ITPA/DDRGI1 variants reflecting thrombocytopenia in pegylated interferon and ribavirin therapy for chronic hepatitis C. *Hum Mol Genet* 2011;20:3507–3516.
- [65] Thompson AJ, Clark PJ, Singh A, Ge D, Fellay J, Zhu M, et al. Genome-wide association study of interferon-related cytopenia in chronic hepatitis C patients. *J Hepatol* 2012;56:313–319.
- [66] Zeuzem S, Soriano V, Asselah T, Bronowicki JP, Lohse AW, Mullhaupt B, et al. Faldaprevir and telaprevir for HCV genotype 1 infection. *N Engl J Med* 2013;369:630–639.
- [67] Fried MW, Buti M, Dore GJ, Flisiak R, Ferenci P, Jacobson I, et al. Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naïve genotype 1 hepatitis C: the randomized PILLAR study. *Hepatology* 2013;58:1918–1929.
- [68] Lawitz E, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, et al. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013;368:1878–1887.
- [69] Kwo P, Gitlin N, Nahass R, Bernstein D, Etzkorn K, Rojter S, et al. Simeprevir plus sofosbuvir (12 and 8 weeks) in hepatitis C virus genotype 1-infected patients without cirrhosis: OPTIMIST-1, a phase 3, randomized study. *Hepatology* 2016;64:370–380.
- [70] Meissner EG, Bon D, Prokunina-Olsson L, Tang W, Masur H, O'Brien TR, et al. IFNL4-DeltaG genotype is associated with slower viral clearance in hepatitis C, genotype-1 patients treated with sofosbuvir and ribavirin. *J Infect Dis* 2014;209:1700–1704.
- [71] Chu TW, Kulkarni R, Kane EJ, Roberts SK, Stedman C, Angus PW, et al. Effect of IL28B genotype on early viral kinetics during interferon-free treatment of patients with chronic hepatitis C. *Gastroenterology* 2012;142:790–795.
- [72] Peiffer KH, Sommer L, Susser S, Vermehren J, Hermann E, Doring M, et al. Interferon lambda 4 genotypes and resistance-associated variants in patients infected with hepatitis C virus genotypes 1 and 3. *Hepatology* 2016;63:63–73.
- [73] Walker A, Skibbe K, Steinmann E, Pfaender S, Kuntzen T, Megger DA, et al. Distinct escape pathway by hepatitis C virus genotype 1a from a dominant CD8+ T cell response by selection of altered epitope processing. *J Virol* 2016;90:33–42.
- [74] Timm J, Lauer GM, Kavanagh DG, Sheridan I, Kim AY, Lucas M, et al. CD8 epitope escape and reversion in acute HCV infection. *J Exp Med* 2004;200:1593–1604.
- [75] Ruhl M, Knuschke T, Schewior K, Glavinic L, Neumann-Haefelin C, Chang DI, et al. CD8+ T-cell response promotes evolution of hepatitis C virus nonstructural proteins. *Gastroenterology* 2011;140:2064–2073.
- [76] Wright M, Goldin R, Fabre A, Lloyd J, Thomas H, Trepo C, et al. Measurement and determinants of the natural history of liver fibrosis in hepatitis C virus infection: a cross sectional and longitudinal study. *Gut* 2003;52:574–579.
- [77] Eslam M, Khattab MA, Harrison SA. Insulin resistance and hepatitis C: an evolving story. *Gut* 2011;60:1139–1151.
- [78] Loomba R, Schork N, Chen CH, Bettencourt R, Bhatt A, Ang B, et al. Heritability of hepatic fibrosis and steatosis based on a prospective twin study. *Gastroenterology* 2015;149:1784–1793.
- [79] Bochud PY, Bibert S, Kutalik Z, Patin E, Guernon J, Nalpas B, et al. IL28B alleles associated with poor hepatitis C virus (HCV) clearance protect against inflammation and fibrosis in patients infected with non-1 HCV genotypes. *Hepatology* 2012;55:384–394.
- [80] Eslam M, Hashem AM, Leung R, Romero-Gomez M, Berg T, Dore GJ, et al. Interferon-lambda rs12979860 genotype and liver fibrosis in viral and non-viral chronic liver disease. *Nat Commun* 2015;6:6422.
- [81] Rembeck K, Alsio A, Christensen PB, Farkkila M, Langeland N, Buhl MR, et al. Impact of IL28B-related single nucleotide polymorphisms on liver histopathology in chronic hepatitis C genotype 2 and 3. *PLoS One* 2012;7:e29370.
- [82] Probst A, Dang T, Bochud M, Egger M, Negro F, Bochud PY. Role of hepatitis C virus genotype 3 in liver fibrosis progression—a systematic review and meta-analysis. *J Viral Hepat* 2011;18:745–759.
- [83] van der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012;308:2584–2593.
- [84] Grebely J, Page K, Sacks-Davis R, van der Loeff MS, Rice TM, Bruneau J, et al. The effects of female sex, viral genotype, and IL28B genotype on spontaneous clearance of acute hepatitis C virus infection. *Hepatology* 2014;59:109–120.
- [85] Lagging M, Askarieh G, Negro F, Bibert S, Soderholm J, Westin J, et al. Response prediction in chronic hepatitis C by assessment of IP-10 and IL28B-related single nucleotide polymorphisms. *PLoS One* 2011;6:e17232.
- [86] Noureddin M, Wright EC, Alter HJ, Clark S, Thomas E, Chen R, et al. Association of IL28B genotype with fibrosis progression and clinical outcomes in patients with chronic hepatitis C: a longitudinal analysis. *Hepatology* 2013;58:1548–1557.
- [87] Bruno S, Thompson AJ, Critelli R, Crosignani A, Rossi S, De Lisi S, et al. Interferon lambda-3 is not associated with clinical outcome in patients with HCV-induced compensated cirrhosis: a long-term cohort study. *Antiviral Res* 2015;113:27–32.
- [88] Fabris C, Falletti E, Cussigh A, Bitetto D, Fontanini E, Bignulin S, et al. IL-28B rs12979860 C/T allele distribution in patients with liver cirrhosis: role in the course of chronic viral hepatitis and the development of HCC. *J Hepatol* 2011;54:716–722.
- [89] Sato M, Kato N, Tateishi R, Muroyama R, Kowatari N, Li W, et al. IL28B minor allele is associated with a younger age of onset of hepatocellular carcinoma in patients with chronic hepatitis C virus infection. *J Gastroenterol* 2014;49:748–754.
- [90] Chang KC, Tseng PL, Wu YY, Hung HC, Huang CM, Lu SN, et al. A polymorphism in interferon L3 is an independent risk factor for development of hepatocellular carcinoma after treatment of hepatitis C virus infection. *Clin Gastroenterol Hepatol* 2015;13:1017–1024.
- [91] Eurich D, Boas-Knoop S, Bahra M, Neuhaus R, Somasundaram R, Neuhaus P, et al. Role of IL28B polymorphism in the development of hepatitis C virus-induced hepatocellular carcinoma, graft fibrosis, and posttransplant antiviral therapy. *Transplantation* 2012;93:644–649.
- [92] Eslam M, George J. Genome-Wide Association Studies and Hepatitis C: Harvesting the Benefits of the Genomic Revolution. *Semin Liver Dis* 2015;35:402–420.
- [93] Patin E, Kutalik Z, Guernon J, Bibert S, Nalpas B, Jouanguy E, et al. Genome-wide association study identifies variants associated with progression of liver fibrosis from HCV infection. *Gastroenterology* 2012;143:1244–1252, e1–e12.
- [94] Urabe Y, Ochi H, Kato N, Kumar V, Takahashi A, Muroyama R, et al. A genome-wide association study of HCV-induced liver cirrhosis in the Japanese population identifies novel susceptibility loci at the MHC region. *J Hepatol* 2013;58:875–882.

## Review

- [95] Kumar V, Kato N, Urabe Y, Takahashi A, Muroyama R, Hosono N, et al. Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. *Nat Genet* 2011;43:455–458.
- [96] Miki D, Ochi H, Hayes CN, Abe H, Yoshima T, Aikata H, et al. Variation in the DEPDC5 locus is associated with progression to hepatocellular carcinoma in chronic hepatitis C virus carriers. *Nat Genet* 2011;43:797–800.
- [97] Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008;40:1461–1465.
- [98] Valenti L, Al-Serri A, Daly AK, Galmozzi E, Rametta R, Dongiovanni P, et al. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2010;51:1209–1217.
- [99] Liu YL, Patman GL, Leathart JB, Piguet AC, Burt AD, Dufour JF, et al. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *J Hepatol* 2014;61:75–81.
- [100] Trepo E, Pradat P, Potthoff A, Momozawa Y, Quertinmont E, Gustot T, et al. Impact of patatin-like phospholipase-3 (rs738409 C>G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C. *Hepatology* 2011;54:60–69.
- [101] Eslam M, Mangia A, Berg T, Chan HL, Irving WL, Dore GJ, et al. Diverse impacts of the rs58542926 E167K variant in TM6SF2 on viral and metabolic liver disease phenotypes. *Hepatology* 2016;64:34–46.
- [102] Kozlitina J, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjaerg-Hansen A, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014;46:352–356.
- [103] Liu YL, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JB, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun* 2014;5:4309.
- [104] Milano M, Aghemo A, Mancina RM, Fischer J, Dongiovanni P, De Nicola S, et al. Transmembrane 6 superfamily member 2 gene E167K variant impacts on steatosis and liver damage in chronic hepatitis C patients. *Hepatology* 2015;62:111–117.
- [105] Ghosh Z, Mallick B, Chakrabarti J. Cellular versus viral microRNAs in host-virus interaction. *Nucleic Acids Res* 2009;37:1035–1048.
- [106] Bandiera S, Pfeffer S, Baumert TF, Zeisel MB. MiR-122—a key factor and therapeutic target in liver disease. *J Hepatol* 2015;62:448–457.
- [107] Girard M, Jacquemin E, Munnich A, Lyonnet S, Henrion-Caude A. MiR-122, a paradigm for the role of microRNAs in the liver. *J Hepatol* 2008;48:648–656.
- [108] Jangra RK, Yi M, Lemon SM. Regulation of hepatitis C virus translation and infectious virus production by the microRNA miR-122. *J Virol* 2010;84:6615–6625.
- [109] Henke JI, Goergen D, Zheng J, Song Y, Schuttler CG, Fehr C, et al. MicroRNA-122 stimulates translation of hepatitis C virus RNA. *EMBO J* 2008;27:3300–3310.
- [110] Janssen HL, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, et al. Treatment of HCV infection by targeting microRNA. *N Engl J Med* 2013;368:1685–1694.
- [111] Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorf LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. *Nature* 2009;461:747–753.
- [112] Jakobsdottir J, Gorin MB, Conley YP, Ferrell RE, Weeks DE. Interpretation of genetic association studies: markers with replicated highly significant odds ratios may be poor classifiers. *PLoS Genet*. 2009;5 e1000337.
- [113] Manolio TA. Bringing genome-wide association findings into clinical use. *Nat Rev Genet* 2013;14:549–558.
- [114] Rueger S, Bochud PY, Dufour JF, Mullhaupt B, Semela D, Heim MH, et al. Impact of common risk factors of fibrosis progression in chronic hepatitis C. *Gut* 2015;64:1605–1615.
- [115] Gibson G. Rare and common variants: twenty arguments. *Nat Rev Genet* 2011;13:135–145.
- [116] Trepo E, Romeo S, Zucman-Rossi J, Nahon P. PNPLA3 gene in liver diseases. *J Hepatol* 2016;65:399–412.
- [117] Swerdlow DI, Holmes MV, Harrison S, Humphries SE. The genetics of coronary heart disease. *Br Med Bull* 2012;102:59–77.
- [118] Huang H, Shiffman ML, Friedman S, Venkatesh R, Bzowej N, Abar OT, et al. A 7 gene signature identifies the risk of developing cirrhosis in patients with chronic hepatitis C. *Hepatology* 2007;46:297–306.
- [119] Eslam M, Hashem AM, Romero-Gomez M, Berg T, Dore GJ, Mangia A, et al. FibroGENE: A gene-based model for staging liver fibrosis. *J Hepatol* 2016;64:390–398.
- [120] Mann DA. Epigenetics in liver disease. *Hepatology* 2014;60:1418–1425.
- [121] Murphy TM, Mill J. Epigenetics in health and disease: heralding the EWAS era. *Lancet* 2014;383:1952–1954.
- [122] Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* 2003;33:245–254.
- [123] Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, et al. Global variation in copy number in the human genome. *Nature* 2006;444:444–454.