

# Review article: genetic factors that modify the outcome of viral hepatitis

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## SUMMARY

### Background

Genetic factors can play an important role for treatment response and disease progression in chronic viral hepatitis.

### Aim

To review the influence of host genetic factors on the clinical course as well as on treatment response in patients with viral hepatitis.

### Methods

Review of the literature.

### Results

A landmark genome-wide association study (GWAS) identified polymorphisms in the *IL28B* gene on chromosome 19 (19q13.13) associated with response to therapy with pegylated interferon- $\alpha$  (PEG-IFN) and ribavirin (RBV) and spontaneous viral clearance in acute hepatitis C. Furthermore, *IL28B* genotype is associated with changes of lipid metabolism and insulin resistance. A further GWAS demonstrated that *ITPA* genetic variants protect HCV genotype 1 patients from RBV-induced anaemia. Another polymorphism in the patatin-like phospholipase domain containing 3 (*PNPLA3*) is associated with hepatic steatosis. Difficult-to-treat hepatitis C patients homozygous for GG had an up to five-fold lower chance of viral clearance on PEG/RBV than non-GG patients. In chronic hepatitis B patients treated with PEG-IFN several retrospective analyses of *IL28B* rs12980275 and rs12979860 genotypes yielded conflicting results which can be explained by the heterogeneity between the study populations. Some variants of the HLA-DP locus (*HLA-DPA1* A allele and *HLA-DPB1*) protect against progression of chronic hepatitis B infection.

### Conclusions

The determination of *IL28B* polymorphisms may be useful to individualise treatment options when using PEG/RBV based therapies for chronic hepatitis C infection. In contrast, so far identified genetic factors play only a minor role in chronic hepatitis B infection.

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Over the past years, genome-wide association studies (GWAS) allowed to study the associations between mapped single nucleotide polymorphisms (SNP) and the presence of common complex conditions in large patient cohorts, thereby revolutionising the study of many traits and diseases.<sup>1</sup> An important yield of genome-wide association studies is information about the role of specific proteins and biological pathways in pathogenesis; these proteins and pathways are candidate targets for the development of preventive and therapeutic methods for disease management.<sup>2</sup>

Several GWAS in patients with chronic hepatitis C treated with pegylated interferon- $\alpha$  (PEG) and ribavirin (RBV) lead to a better understanding of the effects of interferon and of ribavirin (RBV)-induced anaemia. A GWAS investigating the response pattern to PEG/RBV in genotype (GT) 1 in a randomised-controlled prospective study (IDEAL study<sup>3</sup>) identified a SNP in rs12979860, 3 kilobases upstream of the *IL28B* (interleukin 28B) region, strongly associated with treatment outcome.<sup>4</sup> Patients homozygous for the beneficial C allele had a two- to three-fold higher chance to eradicate the virus under treatment with PEG/RBV than patients carrying the T allele. These data were confirmed by further studies.<sup>5–7</sup> Different GWAS identified additional SNPs (rs8099917 and rs12980275)<sup>8–10</sup> in vicinity of the *IL28B* gene with a strong association with nonresponse to treatment in GT 1. A further GWAS of the IDEAL study demonstrated SNPs genetic variants on chromosome 20 in the rs6051702 region (major allele A) with two functional variants (rs1127354 and rs7270101) which both protect against RBV-reduced anaemia. Similar data were obtained in Japanese patients.<sup>11</sup>

## INTERLEUKIN 28B POLYMORPHISMS AND HEPATITIS C VIRUS (HCV)

### Spontaneous viral clearance

In 2009, the *IL28B* SNP rs12979860 was identified to be strongly associated with spontaneous viral clearance in acute hepatitis C (AHC).<sup>12</sup> Furthermore, another study showed that *IL28B* CC genotype was associated with jaundice and spontaneous resolution of the virus.<sup>13</sup> These data were confirmed by Beinhardt *et al.* and were extended to the observation that serum levels of IP-10 (interferon- $\gamma$  inducible protein-10) increased the predictive value of *IL28B* polymorphism for spontaneous clearance in AHC.<sup>14</sup> Recently Duggal *et al.* described the association of SNP in rs4273729 localised near the HLA class II genes on Chromosome 6 with spontaneous reso-

lution of HCV infection independently of *IL28B*. SNPs *IL28B* and DQB1\*03:01 (HLA class II) may explain approximately 15% of spontaneous resolution.<sup>15</sup> In the same line, G allele of the tapasin gene (an important component of the peptide loading complex for HLA class I) was significantly associated with the outcome of HCV infection.<sup>16</sup>

### Response to anti-viral therapy

At present all effective treatment of genotype (GT) 1 chronic hepatitis C include PEG/RBV. For GT1-infected patients, they were used to be combined with the first generation protease inhibitors telaprevir (TPV) or boceprevir (BOC). Within the last months, two new compounds were approved in the USA and Canada [the NS5B polymerase inhibitor sofosbuvir (SOF) and the 'second wave' protease inhibitor simeprevir (SMV)]. In Europe only Sofosbuvir was approved so far. These DAAs in combination with PEG/RBV will replace BOC and TPV within the foreseeable future in GT1 chronic hepatitis C. Furthermore, SOF was approved for an all-oral regimen with RBV for GT2 and 3.

Predicting treatment response is useful to reduce costs and optimise treatment outcomes. A number of pre-treatment predictors – viral and host – are well established in PEG/RBV treatment regimens with or without protease inhibitors. Besides host factors like age, BMI, sex, ethnicity, fibrosis stage and insulin resistance, genetic factors went more and more into the focus of clinical research within the past years.

*IL28B* resides on the short arm of chromosome 19 (19q13.13) and encodes for interferon (IFN)- $\lambda$ 3. IFN- $\lambda$ 3 belongs, together with *IL28A* (IFN- $\lambda$ 2) and *IL29* (IFN- $\lambda$ 1), to the family of type III interferons and was first described in 2003.<sup>17</sup> Several viral infections are able to induce the expression of IFN- $\lambda$  subtypes, and subsequently agonists of toll-like receptors induce type III IFNs.<sup>18, 19</sup> The expression of type I (IFN- $\alpha$  and - $\beta$ ) and III IFNs is induced by the same agents and regulated by common mechanisms.<sup>20</sup> IFN- $\lambda$ s signal through a unique janus-kinases/signal transducers and activators of transcription (JAK/STAT) with common downstream signalling system with the type I IFNs.<sup>10, 21</sup> The molecular mechanism, how *IL28B* genotype influences the response to anti-viral treatment remains unclear. One possible explanation might be elevated levels of interferon stimulated genes (ISG) in patients with the unfavourable T allele compared to CC carriers.<sup>22, 23</sup> *IL28B* genotype might be associated with a specific cell-type modulation of ISG expression (MxA, PKR, OAS1, ISG15) in hepato-

cytes and peripheral blood mononuclear cells.<sup>24</sup> Furthermore, a recently published article discovered a dinucleotide variant in ss469415590 (TT or ΔG), that is in high linkage disequilibrium with rs12979860,<sup>25, 26</sup> Ss469415590 (ΔG) associated with rs12979860 T allele is a frameshift variant that creates a novel gene *IFNL4*, encoding for IFN-λ4 another member of the type III IFN-family sharing a 40.8% amino sequence similarity with IFN-λ3. The insertion ss469415590 (TT) in *IL28B* CC patients leads to an abrogation of IFN-λ4. In treatment-resistant *IL28B* genotype patients, immune cell infiltration of the liver was impaired and hepatic ISGs were up-regulated mediated by multiple factors, including *IL28A/B*, IFN-λ4 and *WNT5A* (wingless-related MMTV integration site 5A).<sup>27</sup> Moreover, *IFNL4* induces expression of ISG in HepG2 hepatoma cells, which might explain lower response rates in patients with the ΔG genotype. By contrast, a study by Hamming *et al.* identified a potent anti-viral effect of IFN-λ4 against both HCV and coronaviruses.<sup>28</sup> *IFNL4* is strongly associated with SVR in both GT1 and 4, but not in GT2 and 3. Thus, due to its strong correlation with rs12979860 in *IL28B*, it provides no additional information for treatment prediction at least in Caucasian patients. In contrast, *IFNL4* is only moderately correlated with SNP rs8099917 in Caucasian patients and determination of *IFNL4* in the clinical setting is therefore superior.<sup>29, 30</sup> The difference between the two commonly used *IL28B* polymorphisms may be due to their different distances from ss469415590. *IL28B* rs12979860 resides 3 kb upstream of *IFNL3* within intron 1 of *IFNL4* whereas rs8099917 lies 9 kb upstream of *IFNL3* and hence outside of *IFNL4*.<sup>31</sup> In African population, *IFNL4* showed only a weak correlation with rs8099917 and a moderate correlation with rs12979860. With this respect, determination of *IFNL4* genotype in patients with African ancestry might be superior to determination of SNPs in *IL28B*.

*IL28B* genotypes are differently distributed across different ethnicities with highest allele frequencies of the beneficial CC among individuals from Eastern and South-Eastern Asia and lowest among those of African ancestry.<sup>8</sup> This variable distribution might at least partly explain the different SVR rates among different ethnicities.

**Role of *IL28B* in the era of direct anti-viral agents.** In 2011, two direct acting anti-virals (DAA) – boceprevir (BOC) and telaprevir (TPV) – were approved for the treatment of HCV GT1 chronic infection and opened a

new era in the field of CHC therapy.<sup>32</sup> Anti-viral triple therapy raised overall SVR rates in previously untreated patients from 40–45% to 68–79%.<sup>33, 34</sup> A retrospective analysis<sup>35</sup> from the SPRINT-2 study focused on the relationship between *IL28B* polymorphism and treatment outcome of triple therapy with BOC in treatment-naïve patients. High and equivalent SVR rates were observed in CC patients in both – BOC and PEG/RBV – treatment arms. CC patients were more likely to be eligible for shorter treatment duration than patients with T allele (89% vs. 52%). Ninety-seven per cent of patients with CC achieved a  $\geq 1 \log_{10}$ -drop in HCV-RNA at the end of the 4-week lead-in phase with PEG/RBV, which identifies good responders with high SVR rates and low risk of selection of resistant HCV-variants to BOC. BOC therapy was associated with high SVR improvement in non-CC patients: 55–71% in the BOC arm compared to 27–28% in the PEG/RBV control arm. Overall, logistic regression modelling found the *IL28B* genotype independently associated with SVR in BOC-based therapy, but the effect was attenuated in comparison to PEG/RBV alone. Regardless of the *IL28B* genotype week 4  $\log_{10}$ -drop was the strongest on-treatment predictor of response.

Similar data were obtained in retrospective analysis of a subset of the ADVANCE study population.<sup>36</sup> As in BOC-based triple therapy, the impact of the *IL28B* genotype on SVR was attenuated in the TVR treatment arms. CC patients were more likely to be eligible for shorter treatment duration.

In treatment-experienced patients, *IL28B* is less informative for the outcome of TVR- and BOC-based therapy. Retrospective analysis of the RESPOND-2 and the REALIZE study showed no significant association between *IL28B* and treatment response in either of these studies.<sup>37</sup>

In treatment-naïve GT1 patients treated with a combination of the ‘second wave’ NS3 protease inhibitor simeprevir (SMV) and PEG/RBV within the QUEST-1 study, *IL28B* genotype significantly impacted treatment response.<sup>38</sup> SVR12 rates were significantly higher in patients with CC [94% ( $n = 72/77$ )] than in patients with TC [76% ( $n = 114/150$ )] or TT [65% ( $n = 24/37$ )]. Similar results on SVR12 regarding the *IL28B* were obtained in the QUEST-2 study (treatment-naïve patients) and the PROMISE study (treatment-experienced patients).<sup>39, 40</sup>

The NEUTRINO-study, a single-group open-labelled phase III trial with the nucleoside NS5B polymerase inhibitor sofosbuvir (SOF) plus PEG/RBV in 327 treat-

ment-naïve GT 1, 4, 5 and 6 patients, identified SNP rs12979860 in *IL28B* as independent pre-treatment predictor for SVR. Response rates were 98% ( $n = 93/95$ ) in patients with CC compared to 87% ( $n = 202/232$ ) in patients carrying the T allele (OR: 7.989, CI 95%: 1.815–35.168;  $P = 0.006$ ).<sup>41</sup>

In conclusion, the effect of the *IL28B* genotype on SVR is attenuated in the setting of triple therapy with NS3 protease, NS5A or NS5B polymerase inhibitors. Thus, it is still a useful tool in pre-treatment counselling and for identifying patients eligible for shorter treatment duration.

***IL28B* in IFN-free treatment regimes.** Data from the INFORM-1 study show that *IL28B* genotype might have an effect on early viral kinetics.<sup>42</sup> Patients received the NS5B polymerase inhibitor mericitabine plus the NS3/4A protease inhibitor danoprevir for 13 days. At day 14 the mean reduction in serum HCV-RNA levels was measured. Those patients with *IL28B* CC had a greater decline in HCV-RNA (5.01 log<sub>10</sub> IU/mL) than patients carrying the T allele (4.59 log<sub>10</sub> IU/mL).

SOUND-C2 is to date the largest published study of IFN-free therapy today. It evaluated the combination therapy of the protease inhibitor faldaprevir (BI201335) and the nonnucleoside NS5B polymerase inhibitor deleobuvir (BI207127) with or without RBV in patients with CHC GT 1. Unfortunately, the further development of this treatment was halted. In multivariate analysis GT1b, female sex, normal baseline  $\gamma$ -glutamyl transferase levels and the *IL28B* CC genotype were associated with a higher rate of SVR12.<sup>43</sup> These data suggest that innate immunity and endogenous interferon release may still be important in interferon-free treatment regimes. On the other hand, the effect of *IL28B* might be blunt as more potent agents and combinations with response rates up to almost 100% are on their way.

With the approval of the NS5B polymerase inhibitor sofosbuvir, the first IFN-free combination regimes for treatment of chronic hepatitis C will soon become available. In an all-oral combination of sofosbuvir with daclatasvir (NS5A-inhibitor) high SVR12 rates were achieved in GT1 patients (treatment-naïve or -experienced) and in GT2/3 patients across all *IL28B* genotypes.<sup>44</sup> Similarly, in the AVIATOR-study<sup>45</sup> no differences in SVR rates according to the *IL28B* genotype were observed in both treatment-naïve and -experienced HCV genotype 1 patients: in treatment-naïve patients SVR rates were 98.3% ( $n = 115$ ) for non-CC and 95.3% ( $n = 43$ ) for CC patients, respectively. In prior null-responders SVR rates

were similar across different *IL28B* genotypes: non-CC: 96.3% ( $n = 85$ ) vs. 100% ( $n = 3$ ) in CC carriers. All patients were treated with an all-oral combination therapy with ABT-450/r (protease inhibitor in combination with ritonavir), ABT-267 (NS5A-inhibitor) and/or ABT-333 (nonnucleoside NS5B-inhibitor)  $\pm$  RBV for 12 or 24 weeks. Also in the phase III studies on the combination of Sofosbuvir with ribavirin (POSITRON, FUSION and FISSION) in patients with CHC GT2 and 3 treated with SOF/RBV, no differences in response rates according to the *IL28B* genotype were observed.<sup>39, 46</sup>

On the other hand, a recently published article by Meissner *et al.* provided evidence that IFNL4  $\Delta$ G allele is associated with slower early decline in an IFN-free treatment regime with SOF/RBV in GT1 patients.<sup>47</sup> Due to the low number of patients included, the study was not statistically powered for analysis on SVR. The authors assumed that induced IFN- $\lambda$ 4 expression might lead to hepatic ISG activation, which could have a negative cross-regulatory effect on the immunological response to HCV infection.

#### ***IL28B* and fibrosis progression**

In a recently published study, Nouredin *et al.*<sup>48</sup> observed an association between *IL28B* and fibrosis progression in patients with chronic hepatitis C. In cross-sectional analysis, patients carrying the CC allele had lower Ishak fibrosis scores than T allele carriers. On the other hand they had higher HAI scores, periportal and portal necroinflammation than non-CC patients. Further, CC patients had lower hepatic steatosis.

In longitudinal analysis, there was no difference in fibrosis progression (defined as an increase in Ishak score in two paired liver biopsy of at least 2) between CC and T allele carriers. HAI scores and serum ALT levels improved in patients with CC compared to TC/TT, but without reaching a level of significance. Interestingly, CC carrier had a two-fold higher risk of developing an adverse clinical outcome (defined as death, ascites, spontaneous bacterial peritonitis, variceal haemorrhage, hepatic encephalopathy or hepatocellular carcinoma) than patients with *IL28B* non-CC. The authors concluded, that *IL28B* CC is associated with an state of enhanced anti-viral immune response, which on the one hand leads to higher viral clearance and on the other hand to more necroinflammatory activity. Different proinflammatory, anti-viral cytokines could result in more hepatic inflammation and higher rates of viral clearance in early infection. Conversely, these cytokines might precipitate clinical decompensation in patients with more progres-

sive liver disease. The fact that *IL28B* CC genotype is associated with enhanced anti-viral immune response which promotes both viral clearance and inflammation, but not fibrosis progression suggests different and independent mechanisms for fibrogenesis.

### *IL28B* polymorphisms and metabolic changes in chronic hepatitis C

HCV infection is associated with various interactions with host metabolic pathways. HCV is able to interfere with glucose homeostasis through direct and indirect mechanisms leading to hepatic and extrahepatic insulin resistance (IR) and type 2 diabetes mellitus.<sup>49, 50</sup> Furthermore, steatosis and disorders of lipid metabolism are common in CHC.<sup>51, 52</sup> A recently published study revealed an association between *IL28B* genotype and changes of lipid metabolism<sup>53</sup>: patients with CC had higher levels of total serum cholesterol, apolipoprotein B and low-density lipoprotein (LDL) cholesterol; triglyceride levels were lower in CC than in TC or TT carriers. In addition, a study published by us confirmed these data and found a strong association between *IL28B* T allele and IR.<sup>54</sup> The pathogenetic mechanism remains unclear. Lower levels of ISG in CC patients, as reported previously,<sup>22, 23</sup> might lead to less interference with insulin signalling in hepatocytes.

### Usefulness of *IL28B* determination in clinical practice

The data from the SOUND-C2<sup>43</sup> and the INFORM-1-study<sup>42</sup> point to the fact that innate immune response and endogenous IFN release might still play a role in viral clearance in all-oral IFN-free treatment regimens. Thus, pre-treatment determination of *IL28B* might still have its usefulness, although its effect on treatment response might be attenuated in highly active anti-viral regimes combining two or three different DAA classes. Still, patients carrying the beneficial genotype might benefit from shorter treatment durations. Furthermore, it has to be pointed out that IFN-free treatment regimes will not be affordable in many parts of the world in the near future. It is to be expected that in developing countries and even in most countries of Eastern Europe IFN and RBV will be the backbone of anti-viral treatment for chronic hepatitis C for the next years. The determination of *IL28B* might have its value in individualising therapy to optimise cost effectiveness and furthermore spare side effects.<sup>55</sup> Thus, further prospective studies are in need to evaluate the impact of *IL28B* on treatment success in IFN-free treatments. Establishing predictive factors may allow shortening treatment further allowing reducing the

cost of the new anti-viral treatment. In addition, *IL28B* might be a useful tool to identify those subjects with acute hepatitis C that unlikely will clear the virus spontaneously and would benefit from early therapeutic intervention.<sup>56</sup>

### VITAMIN D RECEPTOR

Vitamin D exerts immunomodulatory effects on the host response against infection with hepatitis HCV.<sup>57</sup> A recent study<sup>58</sup> showed that a common nonsynonymous SNP in the *vitamin D receptor gene (VDR)* (rs2228570 TC), is a predictor of the clinical outcome of PEG-IFN/RBV therapy in GT 1 and 4 patients. This polymorphism causes a threonine-methionine change in the VDR. Minor T allele carriers of *VDR* rs2228570 had a higher probability of obtaining SVR [Odds ratio (OR) = 0.438, CI 95%: 0.204–0.882 *P* = 0.021].

### ADIPONUTRIN (*PNPLA3*) AND CHRONIC HEPATITIS C

Adiponutrin or patatin-like phospholipase domain containing 3 (*PNPLA3*) is a member of the patatin-like phospholipase family. It is expressed in several human tissues with highest expression in the liver.<sup>59, 60</sup> The *in vivo* function of *PNPLA3* in human is widely unknown. *In vitro* studies in Sf9 cells have shown that *PNPLA3* is able to both synthesise and hydrolyse triglycerides. *PNPLA3* acts as a transacylase, which synthesises intracellular triglycerides by transferring acyl groups from monoglycerides to mono- and diglycerides.<sup>61</sup>

In 2008, a GWAS identified a SNP in rs738409 in the *PNPLA3* gene to be associated with non-alcoholic fatty liver disease.<sup>62</sup> The G allele variation, leading to an isoleucine to methionine exchange in amino acid 148 (I148M) of the protein, led to higher liver fat content independent of body mass index, diabetes status, alcohol consumption and ancestry. Furthermore, *PNPLA3* was associated with alcoholic liver disease and alcoholic cirrhosis,<sup>63</sup> as well as with HCC in alcoholic cirrhosis.<sup>64</sup> A different study confirmed these observations, but on the other hand it found only a weak association between *PNPLA3* and HCC in cirrhotic CHC patients.<sup>65, 66</sup>

In Caucasian CHC patients, a study by Trepo *et al.* found a strong and independent association between *PNPLA3* and liver damage.<sup>67</sup> Patients with homozygosity of the risk allele had a 2.5-fold higher risk for hepatic steatosis and an over three-fold higher risk for fibrosis as well as for fibrosis progression. In contrast, no impact on treatment response to PEG/RBV could be observed. In a different study, the effect of *PNPLA3* on steatosis

and fibrosis was missing in GT 3, suggesting distinct pathogenetic mechanisms in GT 3 and GT 1 CHC patients.<sup>68</sup> In GT 3 infection, the viral mechanisms of fat accumulation are probably stronger than the host factors.

A recently published study by Valenti *et al.* found an influence of the risk allele in *PNPLA3* on SVR in difficult-to-cure patients with advanced fibrosis and CHC GT 1 and 4.<sup>69</sup> Non-GG patients had an up to five-fold higher chance of viral clearance under treatment with PEG/RBV than GG patients.

*PNPLA3* genotype might be a useful prognostic factor for individualised treatment in CHC patients. Still, prospective studies are deserved, to evaluate its clinical use, especially in the era of DAA and IFN-free treatment regimens. Further, the pathogenetic mechanisms need to be elucidated in future studies.

### GENETIC FACTORS ASSOCIATED WITH RIBAVIRIN-INDUCED ANAEMIA

RBV is an important component for successful anti-viral treatment in patients chronically infected with hepatitis C (HCV). RBV leads to haemolytic anaemia which is one of the most common side effects of anti-viral treatment.<sup>70</sup> In HCV genotype 1 patients on PEG/RBV treatment, RBV dose reductions (Hb <10 g/dL) are necessary in up to 38% of patients. On treatment, anaemia occurs more often in female than in male patients.<sup>71</sup> In HCV genotype 1 patients on the current standard of care triple therapy, a combination of PEG/RBV and one protease inhibitor (telaprevir or boceprevir), the rate of RBV dose reductions are even higher,<sup>72, 73</sup> whereas anaemia rates are higher in patients treated with telaprevir than boceprevir. In treatment-experienced patients anaemia rates are highest. In the CUPIC<sup>74</sup> trial, erythropoietin was needed in up to 51% of patients and blood transfusions were necessary in 12% of patients. Similar data were observed in Austrian patients<sup>75</sup>: 20% (22/110) of HCV genotype 1 patients with advanced liver fibrosis had severe adverse events, 23% of them due to severe anaemia defined as the need of admission for blood transfusions. This shows the high need to identify patients who will suffer from severe anaemia on anti-viral treatment.

The reasons for RBV-induced anaemia are currently unknown. RBV reduces adenosine triphosphate (ATP) levels in human erythrocytes by depleting guanosine triphosphate (GTP) and subsequently leading to inhibition of the ATP-dependent oxidative metabolism which causes a membrane damage and leads to a premature extravascular red cell removal.<sup>76, 77</sup> In patients with

reduced inosine triphosphate pyrophosphatase (*ITPA*) activity, inosine triphosphate (ITP) accumulates in erythrocytes, which is not a substrate of erythrocyte ATPase.<sup>78–81</sup> Accumulated ITP can substitute for GTP in the generation of adenosine monophosphate (AMP).<sup>82</sup>

### *ITPA* polymorphism

In 2010, a GWAS demonstrated that an *ITPA* genetic variant protects HCV genotype 1 patients on PEG/RBV treatment from RBV-induced anaemia.<sup>83</sup> A SNP on chromosome 20 in the rs6051702 region (major allele A) leads to ITP deficiency and a subsequent protection against RBV-reduced anaemia in the first 4 weeks of anti-viral treatment. Further analysis also identified SNPs on two functional variants carrying the rs6051702 C allele (rs1127354 and rs7270101) that are also highly protective against RBV induced anaemia in European–American HCV GT 1 patients.<sup>76</sup> After these impressive findings, Ochi H. *et al.* showed equal results in Japanese patients.<sup>84</sup> Subsequent analysis identified that this effect persists over the whole treatment period<sup>85</sup> and also applies for HCV genotype 2, 3 and 6 patients,<sup>86, 87</sup> and patients on triple therapy with telaprevir.<sup>88, 89</sup> However, *ITPA* polymorphisms showed no effect on treatment response in patients on PEG/RBV/TVR therapy.<sup>90</sup> Data of patients treated with PEG/RBV/BOC are currently missing. Our own group analysed RBV-induced anaemia according to gender in HCV GT 1 patients on PEG/RBV treatment<sup>91</sup> and demonstrated that the haemoglobin drop is less in young female patients compared to post-menopausal or male patients. This effect is greatest in young female patients with an *ITPA* variant and persists at least until treatment week 24. The reason for this observation is unknown, but one can assume that this might be due to the different hormone status.

### *ITPA* determination in clinical practice

Detecting an *ITPA* variant has become a meaningful tool to evaluate the possibility of anaemia before treatment initiation. This especially applies to experienced patients on triple therapy who should be monitored closely.

### CHRONIC HEPATITIS B

The pathomechanisms of HBV infection are complex.<sup>92</sup> HBV-related liver disease is generally thought to be related to cytotoxic T cell-mediated lysis of infected hepatocytes but complete virus elimination does not occur.<sup>93</sup> The HBV genome replicates by reverse transcription via an RNA intermediate, the pre-genomic RNA. Nucleocapsids with the partially double stranded

HBV-DNA can reenter the hepatocyte nucleus to produce cccDNA or be secreted as complete virions after coating with envelope proteins. The cccDNA has a long half-life and is very resistant to anti-viral therapy, accounting for the difficulty in achieving virus clearance during treatment of chronic hepatitis B. Thus, understanding the mode of action of anti-viral treatments and the study of genetic factors modifying treatment outcome is not fully understood. Currently, treatment of patients with chronic hepatitis B (CHB) is aimed to improve survival by long-term suppression of HBV replication to prevent long-term consequences like liver cirrhosis, hepatic decompensation or hepatocellular carcinoma (HCC). PEG-IFN or nucleos(t)id analogues (NUC) are the first line treatments for patients with chronic hepatitis B.<sup>94</sup> Limited duration of treatment, induction of a sustained off-treatment response due to enhancement of the host's immune response and absence of resistance are advantages of IFN treatment. However, this can be accomplished only in a proportion of CHB patients and in contrast to NUCs interferon treatment is compromised by adverse effects and the uncertainty whether an individual patient will achieve a durable response. Therefore, it is of particular interest to identify factors which are associated with response to PEG-IFN treatment. Age, gender, HBV genotype, baseline ALT levels, interferon-gamma-inducible protein 10 and pre-treatment viral load have been identified as pre-treatment predictors.<sup>95–99</sup> However, these factors do not explain the differences in response to IFN treatment to the whole extent and viral load and transaminases frequently show a fluctuating course during natural history of the disease. Thus, identification of host genetic factors modulating treatment outcome may improve the use of PEG-IFN in clinical practice by selecting patients with the highest probabilities of response. Due to difficult treatment endpoints, different phases of disease during HBV infection, heterogeneity of the treatment population data on genetics and HBV infection are inconsistent to some extent.

#### *IL28B* and IFN treatment of chronic hepatitis B

Based on the impact of *IL28B* polymorphism on the effects of interferon in patients with chronic hepatitis C there was a substantial hope that these effects also might play a role in patients with CHB treated with PEG-IFN. Several retrospective analyses of *IL28B* rs12980275 and rs12979860 genotypes in chronic hepatitis B patients treated with PEG-IFN yielded conflicting results. Some studies identified that the favourable genotype predicts higher on-treatment and off-treatment virological and

serological response both in HBeAg-negative and -positive patients.<sup>100–103</sup> In contrast, other studies did not find significant differences in outcome between variant host genotypes.<sup>104–107</sup>

In a study investigating 205 HBeAg-positive Asian and European CHB patients treated with PEG-IFN with or without lamivudine or standard IFN, HBeAg seroconversion rates at the end of treatment were higher among homozygotes for the favourable *IL28B* genotypes (rs12980275 AA: 51% vs. AG: 26% vs. GG: 10%,  $P < 0.001$ ; rs12979860 CC: 50% vs. CT: 29% vs. TT: 10%,  $P = 0.001$ ).<sup>99</sup> The patients were predominantly male and were chronically infected with HBV genotype C (47%). Importantly, this association of *IL28B* genotype with increased probability of HBeAg seroconversion was apparent at the end of PEG-IFN treatment and during the post-treatment period. In the 182 patients (89%) receiving no LAM or LAM for up to 52 weeks, the beneficial *IL28B* genotypes were also independently associated with an increased probability of HBeAg clearance at 6 months post-treatment [OR for AA vs. AG/GG, 3.54 (95% CI: 1.33–9.41;  $P = 0.008$ ); OR for CC vs. CT/TT, 3.24 (95% CI, 1.21–8.69;  $P = 0.016$ )] also after adjusting for age, HBV genotype, baseline HBV-DNA and ALT levels and previous interferon exposure.<sup>99</sup>

*IL28B* genotype was also independently associated with an increased probability of HBeAg seroconversion through long-term follow-up. HBeAg seroconversion rates were 54%, 35% and 20% in patients with rs12980275 genotype AA, AG and GG ( $P = 0.005$ ), respectively. In a Cox proportional hazards model, rs12980275 genotype AA was associated with a higher probability of HBeAg seroconversion after adjusting for HBV genotype and baseline HBV-DNA and ALT levels (hazard ratio 2.14; 95% CI 1.14–4.31;  $P = 0.018$ ). Similar results were seen for rs12979860. A relationship between *IL28B* genotypes and combined HBeAg seroconversion and HBV-DNA response during long-term follow-up could not be determined. Moreover, after adjustment for HBV genotype A and ethnicity, rs12980275 AA genotype was associated with a higher probability of HBsAg clearance [ $n = 18$  (9%)] after a median of 173 weeks follow-up [HR 3.47 for AA vs. AG/GG (95% CI: 1.04–13.48,  $P = 0.042$ ); for CC vs. CT/TT 2.57 (95% CI, 0.78–9.08;  $P = 0.02$ )]. However, analysing HBeAg seroconversion rates separately according to HBV genotype revealed that HBeAg seroconversion rates were independent of the *IL28B* genotype in HBV genotype D patients.

In 512 HBeAg-positive Chinese CHB patients (55% treated with Peg-IFN $\alpha$ -2a monotherapy and 45% with a

NUC for 12 months), both HBeAg seroconversion rates 6 months after the end of treatment and the percentage of maintained response (defined as normal ALT levels and serum HBV-DNA <500 copies/mL) were lower among patients with rs8099917 TT compared to non-TT patients (29% vs. 52%,  $P = 0.003$ ).<sup>102</sup> A further study analysed the role of *IL28B* rs12979860 in 96 Australian CHB patients treated with 48 weeks of PEG-IFN.<sup>104</sup> Among 60 HBeAg-positive patients, 27% achieved HBeAg seroconversion with HBV-DNA <2000 IU/mL 6 months after EOT irrespective of the *IL28B* genotype (25% in CC vs. 33% in non-CC patients). Also the overall long-term virological response, the percentage of patients reaching serum HBV-DNA level <2000 IU/mL 24 weeks after end of treatment or the rate of HBsAg after loss 33 months of follow-up in 36 HBeAg-negative patients was independent of the *IL28B* genotype. Similar findings were reported in a European cohort of 95 CHB patients (48% HBeAg-positive) treated with Peg-IFN and adefovir for 1 year and followed up for 24 months.<sup>105</sup>

In a European cohort of 101 HBeAg-negative patients (predominantly genotype D) treated with either standard or pegylated-IFN-alpha for a median of 23 months (range, 10–48) and followed up for a median of 11 years (range, 1–17) *IL28B* rs12979860 genotype CC patients were shown to have higher EOT and off-treatment response than non-CC patients.<sup>101</sup> The rate of serum HBsAg clearance was 29% in CC patients compared to 13% in non-CC patients ( $P = 0.039$ ) and genotype CC (OR, 3.9; 95% CI: 1.1–13.2;  $P = 0.025$ ) independently predicted HBsAg clearance.

The most likely explanation of these conflicting results in CHB is the heterogeneity between the study populations, having diverse genetic backgrounds, sample size, treatment regimens and duration of therapy as well as length of follow-up.

Taken together the data, in contrast to chronic hepatitis C *IL28B* genotypes seem to be of less importance in predicting responsiveness to the immunomodulatory effects of pegylated-IFN in chronic hepatitis B. However, in combination with other predictors of response (e.g. viral genotype, HBsAg decline) it can be a valuable additional tool to estimate the likelihood of response. To date, the limited utility of multiple SNPs polymorphisms for predicting interferon treatment outcome for CHB patients does not recommend its application or to define the pre-treatment probability of a response at an individual level.<sup>94</sup>

## HLA

Some variants of the HLA-DP locus (*HLA-DPA1* A allele and *HLA-DPBI*) protect against progression of CHB<sup>108–110</sup> and development of hepatocellular carcinoma,<sup>111</sup> at least in Asian patients. Furthermore, in Asian patients treated with PEG-IFN *HLA-DPA1* genotype was associated with higher HBeAg seroconversion rates.<sup>108, 112</sup> *HLA-DPBI* is a member of the major histocompatibility complex (MHC) class II molecules, which are glycoproteins attached to the cell membrane of predominantly B-lymphocytes, dendritic cells and macrophages. It is currently not known whether these polymorphisms are also protective against HBV chronicity in Caucasian patients. In contrast to Caucasians, in Asian patients the A allele of *HLA-DPBI* is the major and the G allele is the minor allele. These molecules are involved in antigen presentation to CD4<sup>+</sup> helper T-lymphocytes, which, after recognition of the antigen presented, lead to an antibody response.<sup>113</sup>

Further studies are needed to identify other genetic factors modulating treatment response and disease progression and development of hepatocellular carcinoma in CHB patients. Genome-wide association studies in large patient cohorts are ongoing currently. Some preliminary associations were reported already.<sup>114</sup>

In conclusion, genetic factors have an important influence on the evolution and treatment of chronic hepatitis C. Most of these genetic polymorphisms have not entered the clinical practice yet but allow a better understanding of the pathophysiology of chronic HCV infection. Even in the era of the expected interferon/ribavirin-free treatment options genetic factors may modify treatment responses and may allow an individualisation of the length treatment. In contrast, in chronic hepatitis B so far identified genetic factors play only a minor role.

## AUTHORSHIP

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