

# IL28B POLYMORPHISMS IN INTERFERON-TREATED PATIENTS WITH CHRONIC HEPATITIS B

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## ABSTRACT

Pegylated interferon (Peg-IFN) may achieve a sustained off-treatment response in 20-30% of the patients with chronic hepatitis B (CHB). However, given the high cost of treatment, the frequent side effects, and the lack of effectiveness in a large proportion of patients, there have been attempts to identify the subjects who are most likely to benefit with such therapy. Response rates may be significantly increased by careful selection of patients based upon baseline serum alanine aminotransferase, HBV DNA levels, and viral genotype. Recently, genome-wide association studies identified polymorphisms of the interleukin 28B (IL28B) gene as a potent predictor of sustained viral response in chronic hepatitis C patients treated with Peg-IFN plus Ribavirin, encouraging similar studies in HBV. Overall, these studies failed to provide convincing evidences that IL28B genotype significantly impacts on response to Peg-IFN in chronic hepatitis B (CHB) patients, though these studies are very heterogeneous in terms of patient populations, methodology, baseline features, treatment regimens, duration of follow-up, and ethnicity, making new studies in larger cohorts very much needed.

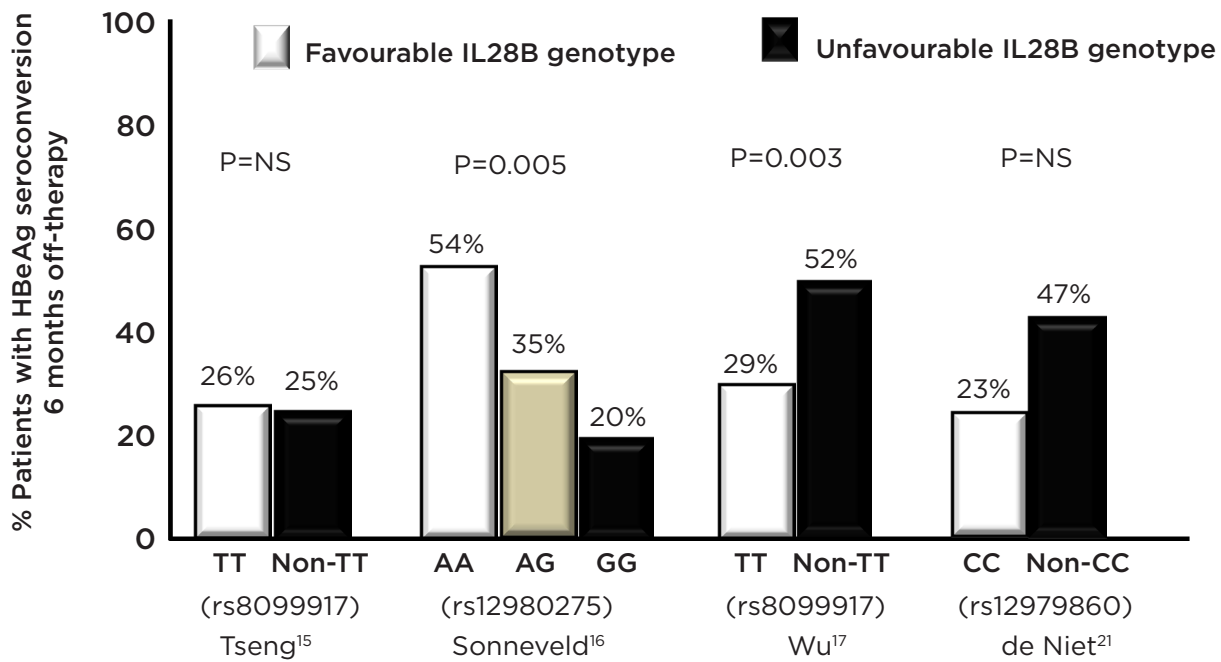
**Keywords:** Chronic hepatitis B, pegylated interferon alpha, sustained response, HBsAg clearance, IL28B polymorphism, rs12979860, rs8099917.

## INTRODUCTION

Antiviral therapy of chronic hepatitis B (CHB) patients is aimed to improve quality of life and survival by preventing progression of liver damage to cirrhosis, hepatic decompensation, hepatocellular carcinoma (HCC), and death.<sup>1-3</sup> This goal can be achieved if hepatitis B virus (HBV) replication can be suppressed in a sustained manner, either by short-term treatment with pegylated interferon (Peg-IFN), or long-term therapy with entecavir and tenofovir.<sup>4</sup> The main advantages of Peg-IFN over nucleoside/nucleotide analogues (NUC) are the absence of resistance, the immunomodulatory properties that induce a direct inhibition of viral replication, and the enhancement of the host's antiviral immune response.<sup>5-9</sup> The major hindrances to the wide usage of Peg-IFN are the need for parenteral therapy and the clinical and laboratory monitoring, its side-

effects profile, and the lack of effectiveness in a large proportion of patients.

To increase its cost-effectiveness, several strategies have been suggested; such as a pre-treatment selection of ideal candidates based upon low viral load, high alanine transaminase (ALT) levels, and viral genotype. However, this approach may be cumbersome as biochemical and virological markers tend to fluctuate over time in HBV-infected patients, viral genotype is not routinely performed in all centres, and more importantly, these variables systematically failed to identify good responders at individual level. Assessment of early on-treatment hepatitis B surface antigen (HBsAg) kinetics is currently the best predictor of non-response to Peg-IFN, as patients with minimal or zero decline of HBsAg or HBsAg levels >20,000 IU/ml have no chance of achieving a sustained response, making Peg-IFN withdrawal



**Figure 1. IL28B polymorphisms and interferon (IFN) response in HBeAg-positive chronic hepatitis B (CHB) patients**

the best option in these patients. However, even this strategy has some disadvantages as 20% of patients receive Peg-IFN for three months without any chance of response, HBsAg quantification is not yet routinely performed in many centres, and good responders cannot be accurately identified.

As far as baseline predictors of response are concerned, a recent major breakthrough was the finding that variation in the region of the interleukin-28B (IL28B) gene is highly predictive of both sustained virological response (SVR) to Peg-IFN and ribavirin in chronic hepatitis C (CHC) patients, and spontaneous HCV clearance in the acute hepatitis C setting.<sup>10-14</sup> These findings have also generated a lot of interest in CHB with the aim to select for only those patients with a high probability of success with interferon treatment.

### IL28B AND INTERFERON RESPONSE IN HBEAG POSITIVE PATIENTS

In a Taiwanese study of 115 HBeAg-positive patients treated with Peg-IFN $\alpha$ -2a for six or twelve months; HBV genotype, major sequences of pre-core (PC) stop codon, basal core promoter (BCP), IL28B (rs8099917) in addition to two genetic variations of HLA-DPA1 (rs3077) and HLA-DPB1 (rs9277535), were genotyped.<sup>15</sup> At the sixth month, post-therapy, patients with the HLA-DPA1 rs3077 GG genotype had a higher HBeAg seroconversion rate than those with

non-GG genotype (35% vs. 13%,  $p=0.007$ ), whereas there were no differences between TT and non-TT IL28B rs8099917 and between GG and non-GG HLA-DPB1 rs9277535 genotypes (26% vs. 25%,  $p=0.92$  and 33% vs. 19%,  $p=0.09$ , respectively) (Figure 1). By multivariate analysis, excluding rs9277535 because it is in high linkage disequilibrium with rs3077, BCP mutation (OR 8.04, 95%CI 2.00-32.28) and the rs3077 GG genotype (OR 3.49, 95%CI 1.12-10.84) were independently associated with a higher HBeAg seroconversion rate. BCP mutation (OR 9.28, 95%CI 1.92-44.99), low baseline viral load (OR 4.78, 95%CI 1.37-16.69), genotype B (OR 5.74, 95%CI 1.06-31.00) and ALT levels >5 upper limit of normal (ULN) (OR 3.72, 95%CI 1.08-12.78) were independently associated with higher combined off-treatment response rates, defined as HBeAg seroconversion, HBV DNA <20,000 IU/mL and ALT normalisation.

In a multicentre study involving 11 Asian and European sites, 205 HBeAg-positive CHB patients treated with either Peg-IFN monotherapy (41%), Peg-IFN+lamivudine (LMV) (52%) or with standard interferon (7%) were retrospectively analysed for IL28B rs12980275 and rs12979860 genotypes.<sup>16</sup> At the end of treatment (EOT), 90 patients (44%) achieved HBeAg seroconversion, with significantly higher rates among rs12980275 AA genotype compared to AG and GG (51% vs. 26% vs. 10%,  $p<0.001$ ), with similar rates observed for rs12979860 CC/CT/TT genotypes (50% vs. 29% vs. 10%,

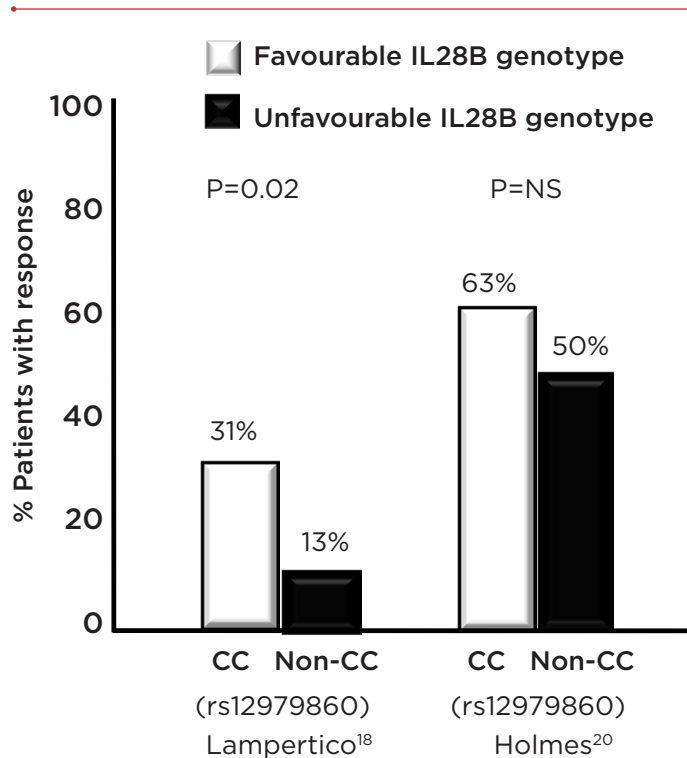
$p < 0.001$ ). IL28B genotypes were also independently associated with an increased probability of HBeAg clearance at six months post-treatment among the 182 patients who received no LMV or LMV for  $\leq 52$  weeks, with an adjusted OR of 3.54 (95%CI 1.33–9.41,  $p = 0.008$ ) for rs12980275 genotype AA versus AG/GG and OR of 3.24 (95%CI 1.21–8.69,  $p = 0.016$ ) for rs12979860 CC versus CT/TT, after adjustment for age, HBV genotype, HBV DNA, ALT levels and previous interferon exposure.

IL28B rs12980275 genotype AA and rs12979860 genotype CC appeared to be associated with a higher probability of HBeAg clearance with HBV DNA level  $< 2,000$  IU/mL, although associations were not significant (OR 2.09 95%CI 0.76–5.75,  $p < 0.139$  for AA vs. AG/GG; OR 1.92 95%CI 0.70–5.26,  $p < 0.188$  for CC vs. CT/TT). 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, respectively ( $p = 0.005$ ) (Figure 1). In a Cox proportional hazards model, genotype AA was associated with a higher probability of HBeAg seroconversion (HR 2.14; 95%CI 1.14–4.31,  $p = 0.018$ ) when adjusting for HBV genotype and baseline HBV DNA and ALT levels. Moreover, after adjustment for HBV genotype A, rs12980275 AA genotype was associated with a higher probability of HBsAg clearance (HR 3.47 for AA vs. AG/GG; 95%CI 1.04–13.48,  $p = 0.042$ ).

A study in 512 Chinese HBeAg-positive patients with CHB, treated with Peg-IFN $\alpha$ -2a monotherapy (55%) or with NUC (45%) for twelve months, retrospectively demonstrated that six months after the EOT, the sustained response defined as normal ALT levels, serum HBV DNA  $< 500$  copies/mL and HBeAg seroconversion was lower among TT compared to non-TT IL28B rs8099917 SNPs (29% vs. 52%,  $p = 0.003$ ).<sup>17</sup> Interestingly, the frequency of the G allele of rs8099917 was significantly higher among patients with a sustained response than in the non-responders' group (8.3% vs. 3.9%,  $p = 0.003$ , OR=0.44, 95%CI 0.25–0.79).

## IL28B AND INTERFERON RESPONSE IN HBEAG NEGATIVE PATIENTS

One Italian study evaluated the role of IL28B in 101 HBeAg-negative patients treated with either standard interferon (68%) or Peg-IFN alfa-2a, for a median of twenty-three months and followed-up for eleven years after treatment.<sup>18</sup> Patients with IL28B



**Figure 2. IL28B polymorphisms and interferon IFN response in HBeAg-negative chronic hepatitis B (CHB) patients**

rs12979860 genotype CC were shown to have higher EOT (69% vs. 45%,  $p = 0.01$ ) and higher sustained virological response, defined as HBV DNA  $< 2,000$  IU/mL (31% vs. 13%,  $p = 0.02$ ), than non-CC patients (Figure 2). Interestingly, CC patients had a higher cumulative probability of clearing HBsAg during long-term follow-up (29% vs. 13%,  $p = 0.04$ ). The IL28B CC genotype was shown to be an independent predictor of sustained virological response together with age and baseline HBV DNA levels, and of serological responses together with baseline HBV DNA and ALT levels, and with the duration of therapy.

To further identify any possible association between IL28B polymorphisms and HBV genotype, the IL28B re-analysis in 93 genotype D patients of the latter study, confirmed that the rates of EOT response, the sustained virological response and the HBsAg clearance were still significantly higher in CC than in non-CC carriers, i.e. 69% vs. 44% ( $p = 0.014$ ), 31% vs. 12% ( $p = 0.028$ ) and 29% vs. 12% ( $p = 0.048$ ). When the assessment of the IL28B polymorphism was extended to include rs8099917, which was shown to improve the prediction of a response to Peg-IFN and RBV therapy in CHC patients with the CT rs12979860 genotype, the favourable rs8099917 TT genotype was found in 100% of the rs12979860 CC patients, compared with only 31% of CT and 10% of

TT patients. The 42 rs12979860 CT patients with the rs8099917 TT genotype had a significantly higher rate of sustained virological response and HBsAg seroclearance (23% vs. 3%,  $p=0.045$  and 23% vs. 0%, respectively  $p=0.007$ ),<sup>19</sup> suggesting that multiple IL28B polymorphisms may be required to accurately define the pretreatment probability of a response at an individual level.

## IL28B AND INTERFERON RESPONSE IN MIXED POPULATION STUDIES

Holmes et al. retrospectively analysed the role of IL28B rs12979860 in 96 Australian CHB patients (62% HBeAg-positive) treated with forty-eight weeks of Peg-IFN and followed up for thirty-nine months.<sup>20</sup> Among 60 HBeAg-positive patients, 27% achieved HBeAg seroconversion with HBV DNA  $<2,000$  IU/mL, six months after EOT without significant difference in response according to IL28B genotype (25% in CC patients vs. 33% in non-CC patients,  $p=0.45$ ) (**Figure 1**). Also, the rate of patients having a serum HBV DNA level  $<2,000$  IU/mL at twenty-four weeks after the EOT, among 36 HBeAg-negative patients, was not affected by IL28B genotype (63% in CC patients vs. 50% in non-CC patients,  $p=0.43$ ). The overall long-term virological response of 39%, after thirty-three months of median follow-up, was not influenced by the IL28 genotype as the rate of HBsAg loss.

No association between IL28B rs12979860 genotypes and HBeAg seroconversion or HBsAg clearance was seen in a European cohort of 95 CHB patients (48% HBeAg-positive), who were treated with Peg-IFN and adefovir for one year and followed-up for twenty-four months.<sup>21</sup> 14 out of 46 (30%) HBeAg-positive patients achieved HBeAg seroconversion, without differences according to IL28B genotype (23% for CC compared to 47% of non-CC). 23 out of 49 (47%) HBeAg-negative patients attained HBV DNA levels  $\leq 2,000$  IU/mL in combination with normal ALT, twenty-four months after stopping therapy, without differences according to IL28B genotype (52% for CC compared to 42% of non-CC). Neither HBeAg-positive nor HBeAg-negative patients showed an association between the rs12979860 polymorphism and HBsAg seroconversion (14% for CC compared to 15% of non-CC).

## CONCLUSION

Overall, the aforementioned studies which investigated the relationship between IL28B polymorphisms and the response to interferon in CHB patients have yielded conflicting results.

Some identified that favourable genotype predicts therapeutic response both in HBeAg-negative and HBeAg-positive patients,<sup>16-18,21</sup> as well as the HBsAg clearance.<sup>16,18</sup> One study identified that the unfavourable IL28B-variant was more frequent in the subgroup of responders,<sup>17</sup> while other studies did not find significant differences in outcome between host genotypes.<sup>15,20</sup>

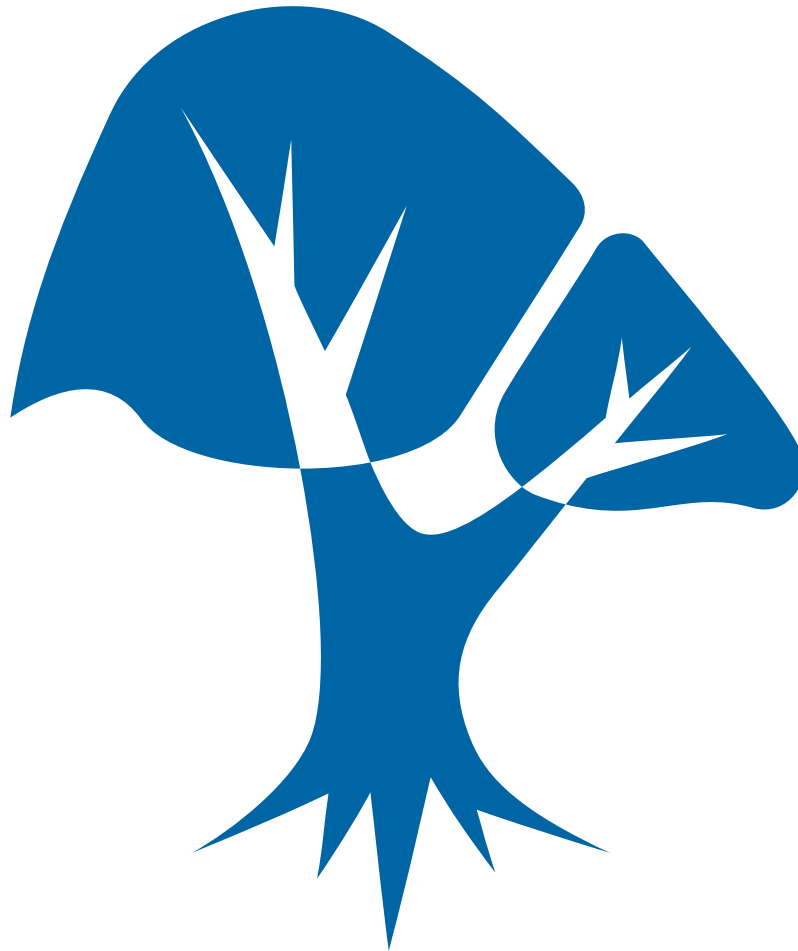
To justify these conflicting results in CHB and in comparison to CHC patients, consideration should be given to the heterogeneity between studies with respect to study populations, diversity of genetic backgrounds, sample size, treatment regimens, and duration of therapy, as well as length of follow-up. Therefore, to conclusively determine that IL28B effects are limited to certain subgroups or may be incidental in small sample-size studies, it is necessary to perform further studies in a large cohort of different ethnic groups to better understand the mechanisms underlying the beneficial effect of this single-nucleotide polymorphism (SNP) in response to interferon treatment. To date, the limited clinical utility for predicting interferon-treatment outcome for CHB patients, does not recommend its application for the selection of patients to start interferon treatment. For now, the success of interferon therapy in CHB patients depends on appropriate patient selection according to HBV DNA and ALT levels and also HBV genotype, but most importantly, on the monitoring of treatment-response based on early on-treatment HBsAg kinetics. Before the genetic test can be implemented into clinical practice, more studies will be needed; such as genome-wide association studies (GWAS), which allow the evaluation of a very large number of patients in informative cohorts, to overcome the previously reported limitations, or by the combination of multiple SNPs to define the pretreatment probability of a response at an individual level. Presently, the use of a genetic marker as a surrogate for the host's ability to clear the virus plays a major role in host immune response to HCV infection but not in HBV.



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