

# Genome-wide Association Study Identifies Genetic Variants Associated With Early and Sustained Response to (Pegylated) Interferon in Chronic Hepatitis B Patients: The GIANT-B Study

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**Background.** (Pegylated) Interferon ([Peg]IFN) therapy leads to response in a minority of chronic hepatitis B (CHB) patients. Host genetic determinants of response are therefore in demand.

**Methods.** In this genome-wide association study (GWAS), CHB patients, treated with (Peg)IFN for at least 12 weeks  $\pm$  nucleos(t)ide analogues within randomized trials or as standard of care, were recruited at 21 centers from Europe, Asia, and North America. Response at 24 weeks after (Peg)IFN treatment was defined as combined hepatitis B e antigen (HBeAg) loss with hepatitis B virus (HBV) DNA  $<2000$  IU/mL, or an HBV DNA  $<2000$  IU/mL for HBeAg-negative patients.

**Results.** Of 1144 patients, 1058 (92%) patients were included in the GWAS analysis. In total, 282 (31%) patients achieved the response and 4% hepatitis B surface antigen (HBsAg) loss. GWAS analysis stratified by HBeAg status, adjusted for age, sex, and the 4 ancestry components identified *PRELID2* rs371991 ( $B = -0.74$ , standard error [SE] = 0.16,  $P = 3.44 \times 10^{-6}$ ) for HBeAg-positive patients. Importantly, *PRELID2* was cross-validated for long-term response in HBeAg-negative patients. *G3BP2* rs3821977 ( $B = 1.13$ , SE = 0.24,  $P = 2.46 \times 10^{-6}$ ) was associated with response in HBeAg-negative patients. *G3BP2* has a role in the interferon pathway and was further examined in peripheral blood mononuclear cells of healthy controls stimulated with IFN $\alpha$  and TLR8. After stimulation, less production of IP-10 and interleukin (IL)-10 proteins and more production of IL-8 were observed with the *G3BP2* G-allele.

**Conclusions.** Although no genome-wide significant hits were found, the current GWAS identified genetic variants associated with (Peg)IFN response in CHB. The current findings could pave the way for gene polymorphism-guided clinical counseling, both in the setting of (Peg)IFN and the natural history, and possibly for new immune-modulating therapies.

**Clinical Trials Registration:** NCT01401400.

**Keywords.** peginterferon; chronic hepatitis B; response; GWAS; genetics.

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Approximately 250 million people worldwide have evidence of a chronic hepatitis B (CHB) infection, which may progress to liver cirrhosis, liver decompensation, hepatocellular carcinoma (HCC), and death. The aim of antiviral therapy is to improve the quality of life and the long-term prognosis [1–3]. Current internationally recommended treatments Tenofovir, Entecavir, and (pegylated) interferon ([Peg]IFN), which can reduce viral load and hepatic necroinflammation, decrease the risk of HCC and complications of cirrhosis [4–6]. (Peg)IFN has both direct antiviral and immune modulating effects. The main advantages of this agent include a finite course of treatment and the lack

of drug resistance. It leads to an improved prognosis and high rates of hepatitis B surface antigen (HBsAg) loss during long-term follow-up in those with a favorable response [7]. However, it requires subcutaneous injections and carries considerable side effects. In addition, only 20–30% of treated patients have a sustained response to treatment [8–11]. It is therefore important to identify host genetic determinants of response to reduce the costs and side effects of treatment and make this treatment modality more acceptable to patients. Genetic host studies on response to (Peg)IFN provide substantial knowledge on the interaction between the host and the virus to induce immune control, both for immune modifying therapy and the natural history of disease. Numerous small studies conducted with selected single-nucleotide polymorphism (SNP) have shown associations with (Peg)IFN response; however, to date, a large genome-wide association study (GWAS) to predict the response to (Peg)IFN in CHB patients has not been performed. Causal or regulating SNPs in genes modifying the immune response can be identified through a GWAS and can be used to assess the chance of response to treatment and select patients who have a high probability of response to (Peg)IFN. This would potentially pave the way for further functional follow-up and clinical validation on gene polymorphisms-guided therapeutic stratification, both in the setting of (Peg)IFN and possibly for new therapeutic agents.

## PATIENTS AND METHODS

### Patients

In this investigator-initiated multicentre global GWA study, CHB patients treated with PegIFN alpha or conventional interferon  $\alpha$  (IFN  $\alpha$ ) within randomized controlled trials, prospective cohort studies, or as part of standard of care at 21 tertiary care centers from Europe, Asia, and North America were initially eligible for inclusion. The inclusion and exclusion criteria for the trials are described elsewhere [7, 8, 10, 12–22]. For the current study, the minimal duration of therapy was 12 weeks. Patients who received combined treatment of PegIFN and nucleos(t)ide analogues (NA) or ribavirin therapy were also included, because combination treatment with these agents does not influence response rates at 6 months post-treatment as compared to PegIFN alone [7–11, 13, 16, 17, 23]. PegIFN add-on therapy to short-term [12] or long-term [14, 20–22] NA treatment was also allowed. Patients were excluded in case of a hepatitis C, hepatitis delta, or human immunodeficiency virus coinfection. An overview of the origin of included patients can be found in [Supplementary Table 1](#). The study was conducted in agreement with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. The study was approved by the ethics committee of each participating centre. All patients gave written informed consent according to standards of the local ethics committees at each of the participating

centres. All authors had access to the study data and reviewed and approved the final manuscript. The study protocol can be accessed at [clinicaltrials.gov](https://clinicaltrials.gov), identifier NCT01401400.

### Endpoints

The primary endpoint was assessed at 24 weeks post (Peg)IFN treatment, and the primary response was defined as combined hepatitis B e antigen (HBeAg) loss with a hepatitis B virus (HBV) DNA <2000 IU/mL for HBeAg-positive patients, and an HBV DNA level <2000 IU/mL for HBeAg-negative patients, including confirmed HBsAg loss, according to standard definitions reported by clinical practice guidelines [1–3]. Other endpoints considered were sustained response (ie, patients with both response at 24 weeks post-(Peg)IFN and at end of follow-up) and end of follow-up response (ie, includes patients who achieved response during follow-up, but excluding those who lost response). Combination of the primary endpoint with ALT normalization was considered as well and can be found in the [Supplementary Materials](#).

### DNA Extraction, Host Genotyping, and Laboratory Measurements

These data can be found in the [Supplementary Materials](#).

### Genome-wide Association and Statistical Analysis

Data were analyzed according to genetic analysis populations (GAP). Patients included in the GAP comprise all patients in the study who were successfully genotyped (ie, passed the quality control steps) and were treated with (Peg)IFN for at least 12 weeks. In this analysis, if data on outcome were missing, patients were not considered in the analysis. GAP analysis was applied both for the primary as well as the secondary endpoints. For each genotyped or imputed SNP, binary logistic regression analysis of the primary response was performed with additive SNP effects for the total cohort with adjustment for sex, age, baseline HBeAg status, and 4 ancestry principal components (PCs). These PCs represent the genetic ethnicity, which are extracted from the genetic data. Next to a GWAS for the total cohort, we performed a stratified GWAS by HBeAg status as well, because patients with HBeAg-negative CHB may be a more genetically selected subgroup and therefore possibly biologically different compared to those with HBeAg-positive CHB [1–3]. RVtests were used for the GWAS analysis [24]. With RVtests, dosage information was used instead of best-guess genotypes. Moreover, all variants with a minor allele frequency <1% were discarded. Finally, with RVtests, the Wald test was used to determine significance [24]. A  $P$ -value of less than  $5 \times 10^{-8}$  was considered genome-wide significant. Q-Q plots were generated to inspect the consistency between the resultant and expected test statistics (ie, any evidence of genomic inflation), and genomic control was applied to adjust for any residual inflation.

### Sensitivity Analyses

We performed a multivariable analysis including the obtained SNPs of interest, additionally adjusted for the duration of (Peg)IFN treatment, HBV DNA load and ALT at baseline. Moreover, a sensitivity analysis for the combination of the primary endpoint with ALT normalization, a stratified analysis by physician-reported ethnicity, and an analysis for the treatment regimen received were performed. Finally, a review of previously described SNPs associated with (PEG)IFN response was performed. All sensitivity analysis can be found in the [Supplementary Materials](#).

## RESULTS

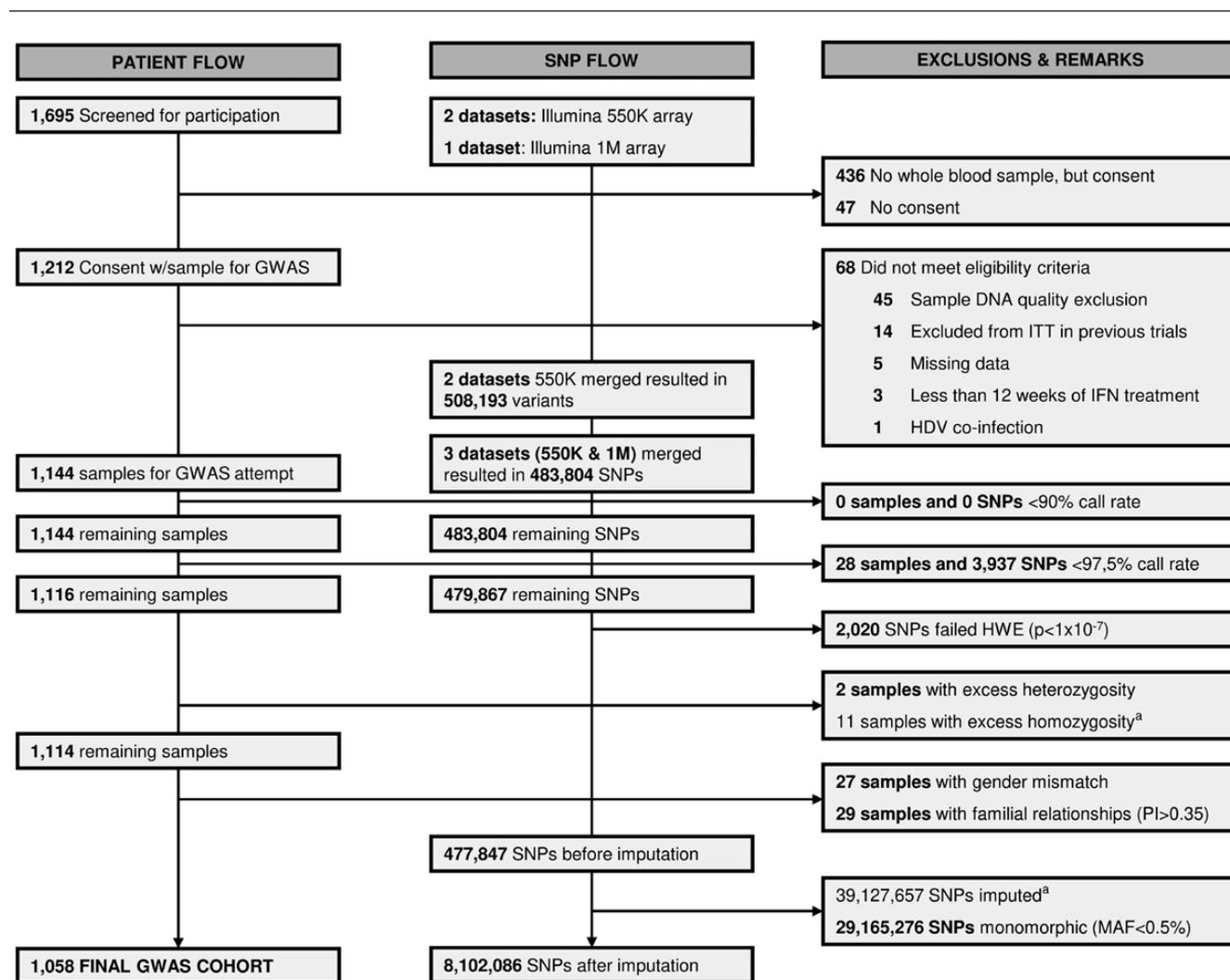
### Patient Characteristics

In total, 1695 patients treated with (Peg)IFN were identified at 21 centers worldwide. The patient selection can be found in the study workflow ([Figure 1](#)). After selection procedures, a GWAS

attempt could be made on 1144 (67%) of which 1058 (92%) were successfully included in the GWAS cohort after quality control checks ([Table 1](#)). In sum, 534 (51%) patients were treated as part of (randomized) trials or per study protocols, and 524 (50%) patients were treated as part of standard of care [[7, 8, 10, 12–22](#)] ([Supplementary Materials](#)). In total, 923 patients were analyzed per GAP for the primary endpoint.

### Primary Outcome and Follow-up

Per GAP analysis, 282 (31%) out of 923 patients achieved the primary response (12% with HBsAg loss, 4% of the GAP cohort). The primary response rate for white patients was 22% (60/278), and 34% (217/636) for Asians. For HBeAg-positive patients the primary response rate was 24% (121/509) and for HBeAg-negative patients 39% (161/414). Patients were followed for a median of 146 weeks (interquartile range [IQR] 96–401) from baseline, which was a median of 99 weeks (IQR 48–353) from cessation of (Peg)IFN treatment.



**Figure 1.** Study workflow for both samples and single-nucleotide polymorphisms. Abbreviations: GWAS, genome-wide association study; HDV, hepatitis Delta virus infection; HWE, Hardy-Weinberg equilibrium; IFN, interferon; ITT, intention-to-treat analysis; MAF, minor allele frequency; PI, PI-HAT, variable calculated by PLINK from the identify-by-state (IBS) matrix; SNPs, single-nucleotide polymorphisms. <sup>a</sup>Not excluded from analysis.

**Table 1. Patient Characteristics According to Hepatitis B e Antigen Status at Baseline**

Characteristics	All Patients (n = 1058)	HBeAg-positive (N = 549)	HBeAg-negative (N = 509)
<b>Demography</b>			
Mean (SD) age, years	39 (11)	35 (10)	43 (10)
Male, n (%)	753 (71%)	374 (68%)	379 (75%)
<b>Reported ethnicity<sup>a</sup></b>			
Asian	646 (61%)	429 (78%)	217 (43%)
White	375 (35%)	101 (18%)	274 (54%)
African/other	37 (3%)	19 (4%)	18 (3%)
Previous IFN therapy, n (%)	88 (8%)	36 (7%)	52 (10%)
<b>Therapy allocation, n (%)</b>			
PegIFN monotherapy	551 (52%)	254 (46%)	297 (58%)
PegIFN + NA	239 (23%)	140 (26%)	99 (20%)
PegIFN add-on	90 (9%)	86 (16%)	4 (1%)
IFN ± NA	156 (15%)	69 (12%)	87 (17%)
PegIFN + Ribavirin	22 (2%)	0	22 (4%)
Duration of (Peg)IFN, weeks	49 (18)	44 (14)	55 (20)
(Peg)IFN as part of study/trial	534 (51%)	308 (56%)	226 (44%)
<b>Laboratory results, mean (SD)</b>			
ALT (U/L) <sup>b</sup>	2.9 (3.0)	3.0 (2.9)	2.8 (3.0)
HBV DNA, log IU/mL	5.7 (2.2)	6.4 (2.2)	5.0 (1.9)
HBsAg, log IU/mL <sup>c</sup>	3.5 (1.0)	3.7 (1.0)	3.2 (0.8)
<b>HBV genotype, n (%)<sup>d</sup></b>			
A	59 (6%)	35 (6%)	24 (5%)
B	78 (7%)	40 (7%)	38 (8%)
C	196 (19%)	102 (19%)	94 (19%)
D	195 (18%)	46 (8%)	149 (29%)
Other	23 (2%)	10 (2%)	13 (3%)
Liver cirrhosis	79 (8%)	26 (5%)	53 (10%)
<b>Long-term follow-up</b>			
Weeks from baseline, median (IQR)	146 (96–401)	144 (77–384)	188 (120–418)
Weeks from EOT, median (IQR)	99 (48–353)	96 (32–347)	135 (72–366)
<b>Primary response GAP, n (%)</b>			
At 24 weeks post IFN	282/923 (31%)	121/509 (24%)	161/414 (39%)
At end of follow-up	261/1028 (25%)	115/534 (22%)	146/494 (30%)
Sustained response <sup>e</sup>	196/902 (22%)	94/497 (19%)	102/405 (25%)

Liver cirrhosis was based on pathology or radiology ± clinical characteristics or the combination of these.

Abbreviations: base, baseline (start (Peg)IFN treatment); EOT, end of treatment; GAP, genetic analysis populations; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; IFN, interferon; IQR, interquartile range; NA, nucleos(t)ide analogue; SD, standard deviation.

<sup>a</sup>Reported ethnicity by local investigator. This does not resemble the ethnicity based on genetics (ancestry principal components) used in the genome-wide association study (GWAS) analyses.

<sup>b</sup>Multiples of upper limit of the normal range.

<sup>c</sup>315 (30%) missing.

<sup>d</sup>507 (48%) missing.

<sup>e</sup>Sustained response was defined as patients with response both at 24 weeks post IFN and at end of follow-up.

### Genome-wide Association Analysis

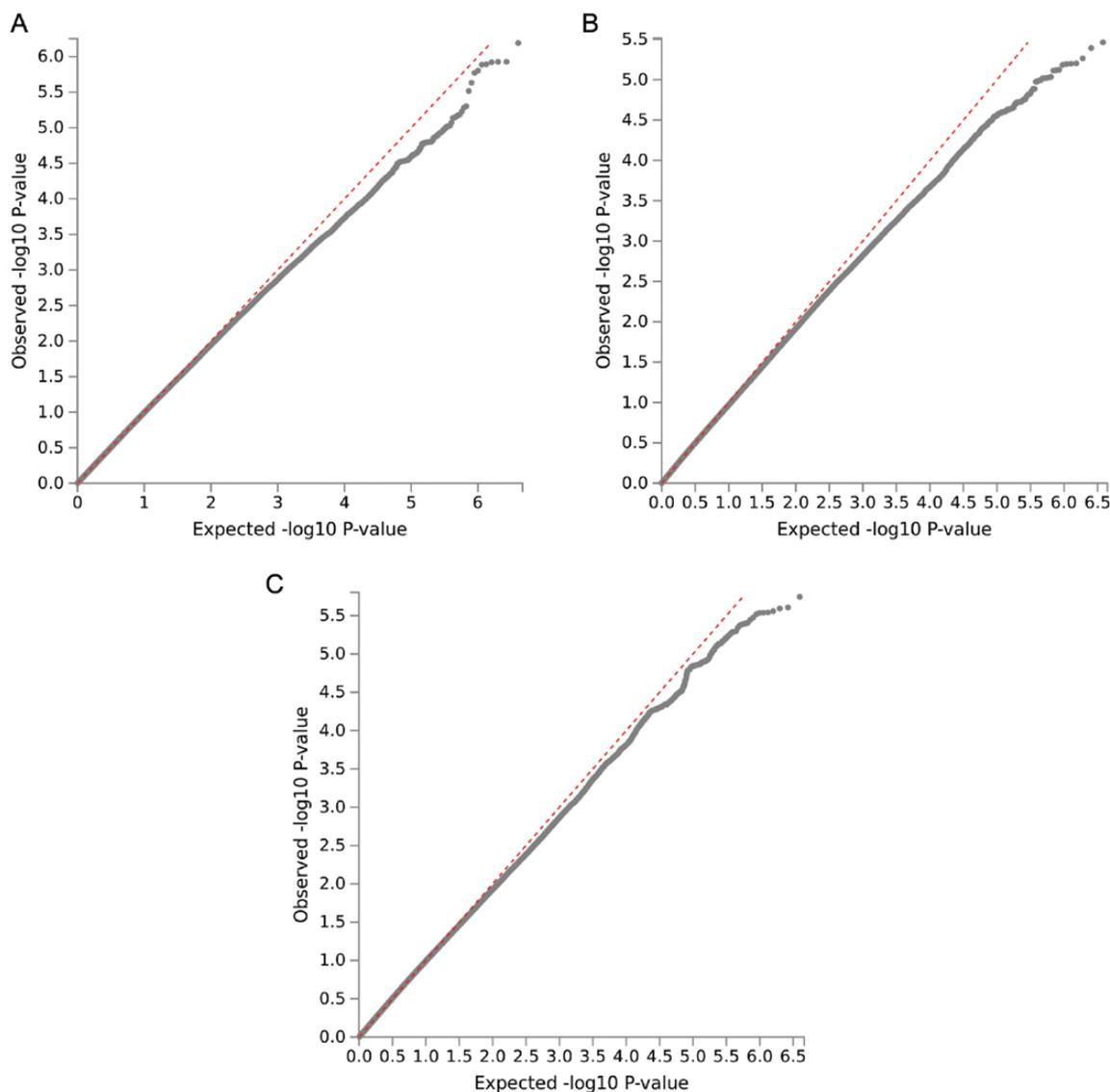
First, a GWAS was performed for the complete cohort (GAP n = 923) adjusted for sex, age, 4 ancestry principal components, and baseline HBeAg status. The Q-Q plot is shown in [Figure 2](#). There were no genome-wide significant associations, but 3 suggestive loci ( $P < 5.0 \times 10^{-6}$ ) were found ([Table 2](#) and [Supplementary Table 2](#)). Of these, *rs78900671* GC allele (*TRAPPC9*, *COL22A1*) had the strongest association (response of best-guess genotypes GG vs GC 28.4% vs 59%; additive model  $\beta = 1.434$ , standard error [SE] = 0.29,  $P = 6.43 \times 10^{-7}$ ). [Figure 3](#) shows the Manhattan plot; for the suggestive loci, zoomed-in versions of the Manhattan plot are illustrated in the

[Supplementary Materials](#). Moreover, both a sensitivity analysis and a look-up of previously described SNPs can be found in the [Supplementary Materials](#) as well.

### Genome-wide Association Analysis Stratified by Baseline HBeAg Status

A subgroup specific GWAS was performed stratified by HBeAg status with adjustment for sex, age, and 4 ancestry principal components. For both groups there were no genome-wide significant associations, but suggestive loci were identified ( $P < 5.0 \times 10^{-6}$ ). The Q-Q and Manhattan plots are shown in [Figures 2](#) and [3](#).

For HBeAg-positive patients, *rs371991* on chromosome 5 (*PRELID2*) was associated with the primary outcome (additive



**Figure 2.** Q-Q plots for the complete cohort (A), HBeAg-positive patients (B) and HBeAg-negative patients (C). Abbreviation: HBeAg, hepatitis B e antigen.

$\beta = -0.74$ ,  $SE = 0.16$ ,  $P = 3.44 \times 10^{-6}$ , Table 2). Response rates for best-guess genotypes were 38%, 23%, and 13% for AA, AG, and GG, respectively (Figure 4). As a sensitivity analysis, a multivariable analysis additionally adjusted for the duration of (Peg) IFN treatment, HBV DNA load, and ALT at baseline, *PRELID2* remained associated with the primary response (additive  $\beta = -0.67$ ,  $SE = 0.16$ , odds ratio [OR] = 0.51,  $P = 3.70 \times 10^{-5}$ ). *PRELID2* was also associated with response at end of follow-up ( $P < .001$ , Figure 4), with sustained response ( $P = .001$ , Figure 4), and with combined HBeAg loss with HBV DNA  $<2000$  IU/mL and ALT normalization ( $P < .001$ , Supplementary Materials) for HBeAg-positive patients.

For HBeAg-negative patients the most interesting top-hit SNP associated with the primary response was *rs3821977* ( $\beta = 1.13$ ,  $SE = 0.24$ ,  $P = 2.46 \times 10^{-6}$ ), which is located on chromosome 4,

intronic within gene *G3BP2*, which has a function within the IFN pathway [25]. Response rates for best-guess genotypes were 15%, 22%, and 49% for AA, AG, and GG, respectively (Figure 4). In sensitivity analysis, this association remained strong after adjustment by the aforementioned variables. *G3BP2* was also associated with sustained response, response at long-term follow-up (Figure 4) and with combined HBV DNA  $<2000$  IU/ml with ALT normalization ( $P < .001$ , Supplementary Materials).

#### ***PRELID2* Cross-validation in HBeAg-negative Patients**

The association of *PRELID2 rs371991* with response in HBeAg-positive patients was further examined in HBeAg-negative patients. Here, we independently validated this association for HBeAg-negative patients at long-term follow-up (sustained response: AA vs AG vs GG: 32%, 26%, 19%; response at end of

**Table 2. Details of Top-hit Loci Associated With the Primary Response for the Total Cohort, HBeAg-positive and HBeAg-negative Patients**

rsID	Chr	Position	Ref.	Eff.	EAF	Beta	SE	P Value	Nearest Genes	Function	Association
<b>Total cohort</b>											
rs78900671	8	139666210	G	C	0.03649	1.434	0.2882	6.43E-07	TRAPPC9 COL22A1	Intronic	Potentiates tumor necrosis factor, alpha-induced NF-kappaB activation. NF-kappaB is a multipotent transcription factor that regulates the expression of numerous genes involved in a wide array of biological responses such as inflammation, immunity, apoptosis, and synaptic plasticity
<b>HBeAg-positive patients</b>											
rs371991	5	145212781	A	G	0.5519	-0.7406	0.1595	3.44E-06	PRELID2	Intronic	PRELI influences Th cell death and Th2 differentiation. PRELI overexpression resulted in STAT6 downregulation. TCR stimulation of naive CD4-positive T cells caused a stimulus dose-dependent increase in PRELI expression coupled with a reduction in CRT2 expression
<b>HBeAg-negative patients</b>											
rs3821977	4	76628351	A	G	0.8304	1.126	0.239	2.46E-06	G3BP2	Intronic	Critical regulator of stress granules assembly Translation of interferon stimulated genes and IFN response pathway Critical role in the regulated secretion of macrophage migration inhibitory factor from monocytes/macrophages

Abbreviations: Beta, effect size; EAF, effect allele frequency; Eff., effect allele; Ref., reference allele; rsID = SNPID, Single nucleotide polymorphism identification

follow-up: 38%, 31%, 23%,  $P = .031$  and  $P = .008$ , respectively, [Figure 4](#)). The association of *G3BP2* with response in HBeAg-negative patients could not be validated upstream the natural history in HBeAg-positive patients ( $P > .05$ ).

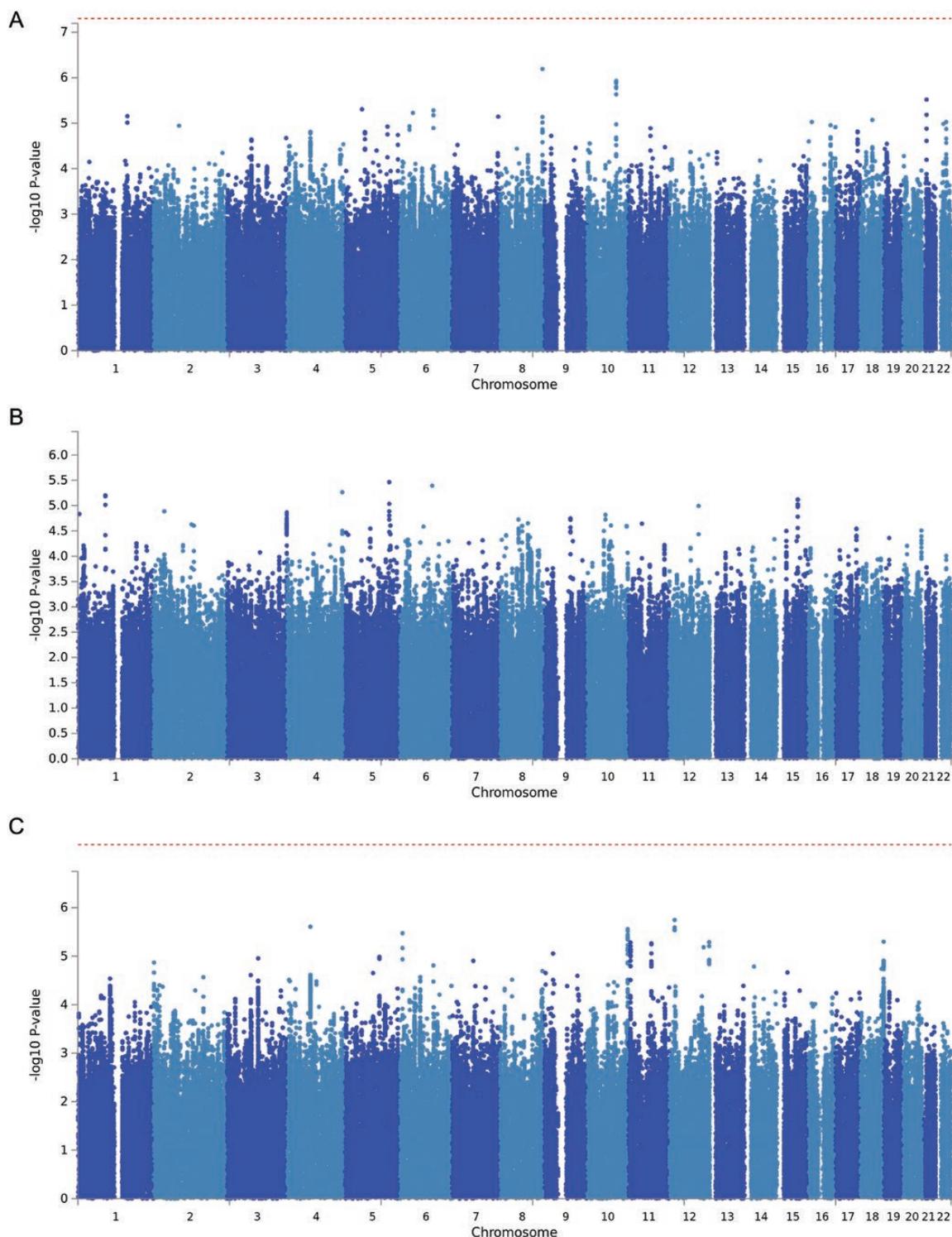
### ***G3BP2* Gene Function Shows a Differential Effect on IL-8, IP-10, and IL-10 Protein Levels**

Because *G3BP2* rs3821977 is described as a factor within the IFN response pathway [25], we further focused on the consequence of the specific SNP genotype on the functionality of blood leukocytes (cytokine production of predominantly monocytes and plasmacytoid dendritic cells) in response to exposure to IFN in healthy controls. The methods for this analysis can be found in the [Supplementary Materials](#). In short, we genotyped healthy controls for the SNP and stimulated peripheral blood mononuclear cells (PBMCs) with IFN alone or in combination with R848 (TLR7/8 ligand). Interleukin (IL)-8, IP-10, and IL-10 showed different trends between groups upon IFN+TLR7/8 stimulation. Although IL-8 was higher in the GG than the AA group, IP-10 and IL-10 exhibited higher levels in the AA group ([Figure 5](#)). For a subset of patients from the GWAS cohort, baseline IP-10 levels were available, which showed no differences across the *G3BP2* genotypes pretreatment ( $P > .5$ ). The results for the other cytokines and in relation to medium or IFN alone, can be found in the [Supplementary Materials](#).

## **DISCUSSION**

In the current global investigator-initiated GWAS, we studied the largest (Peg)IFN treated CHB cohort to date, and identified *TRAPPC9*, *PRELID2* and *G3BP2* to be associated with both short and long-term response to (Peg)IFN. Although not genome-wide significant, these top-hit SNPs were suggestive of a meaningful association ( $P < 10^{-6}$ ), across all ethnicities. Moreover, these SNPs were associated with, or were found within, different immune response pathways. If further corroborated, this may have important implications for both the natural history of CHB and for current PegIFN therapy that should be individualized to patients with the highest likelihood of response. Furthermore, PegIFN is frequently used as an agent to decrease cccDNA and modify the immune response in studies on combination treatment, aiming for functional cure. The outcome of this study may also help us to learn about the biology and likelihood of response to the many other new compounds in development to reach functional cure of CHB.

We performed in a large number of patients 3 GWAS: 1 for the complete group of CHB patients adjusted for HBeAg status, and also 2 separate GWAS stratified by HBeAg. For HBeAg-positive patients, the main finding was the association of *PRELID2* with (Peg)IFN response. This was observed for response at 24 weeks off-treatment, as well as sustained and long-term follow-up response. Further verifying this association, *PRELID2* also showed an association with



**Figure 3.** Manhattan plots for the genome-wide association study for the complete cohort (A), HBeAg-positive patients, (B) and HBeAg-negative patients (C). Abbreviation: HBeAg, hepatitis B e antigen.

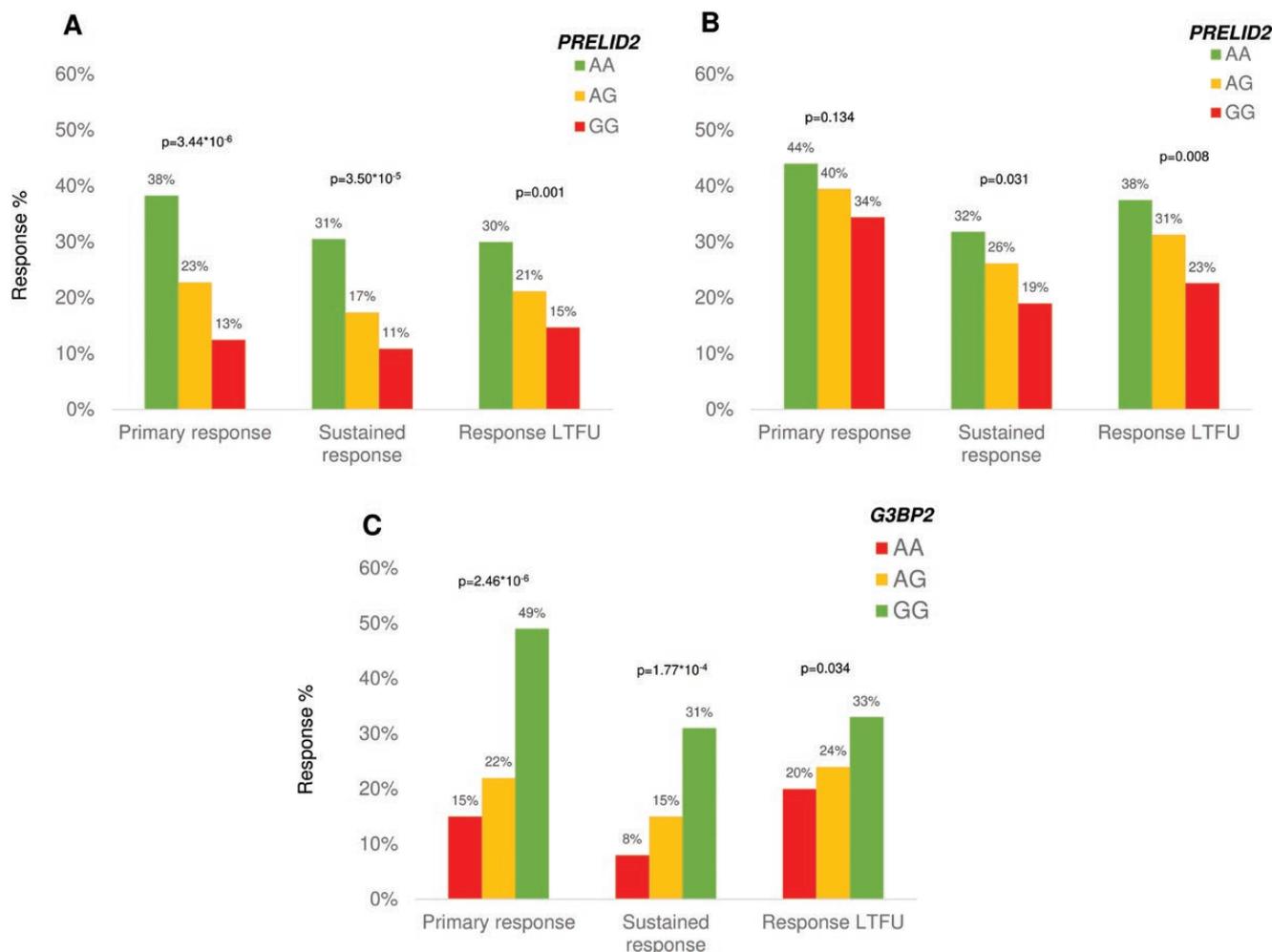
sustained response in an independent cross-validation analysis of HBeAg-negative patients, suggesting this effect is preserved along the natural history of the disease. *PRELID2* is known to downregulate STAT6. Located downstream the IL-4 receptor, lower levels of STAT6 may impair T-helper cell differentiation and T-cell

survival, which are known to be required processes for an effective HBV-specific T-cell response [26, 27]. This suggests a potential role for *PRELID2* in CHB, likely via attenuating T-cell activity. To our knowledge, no other information is currently available on the involvement of *PRELID2* in immune-mediated diseases.

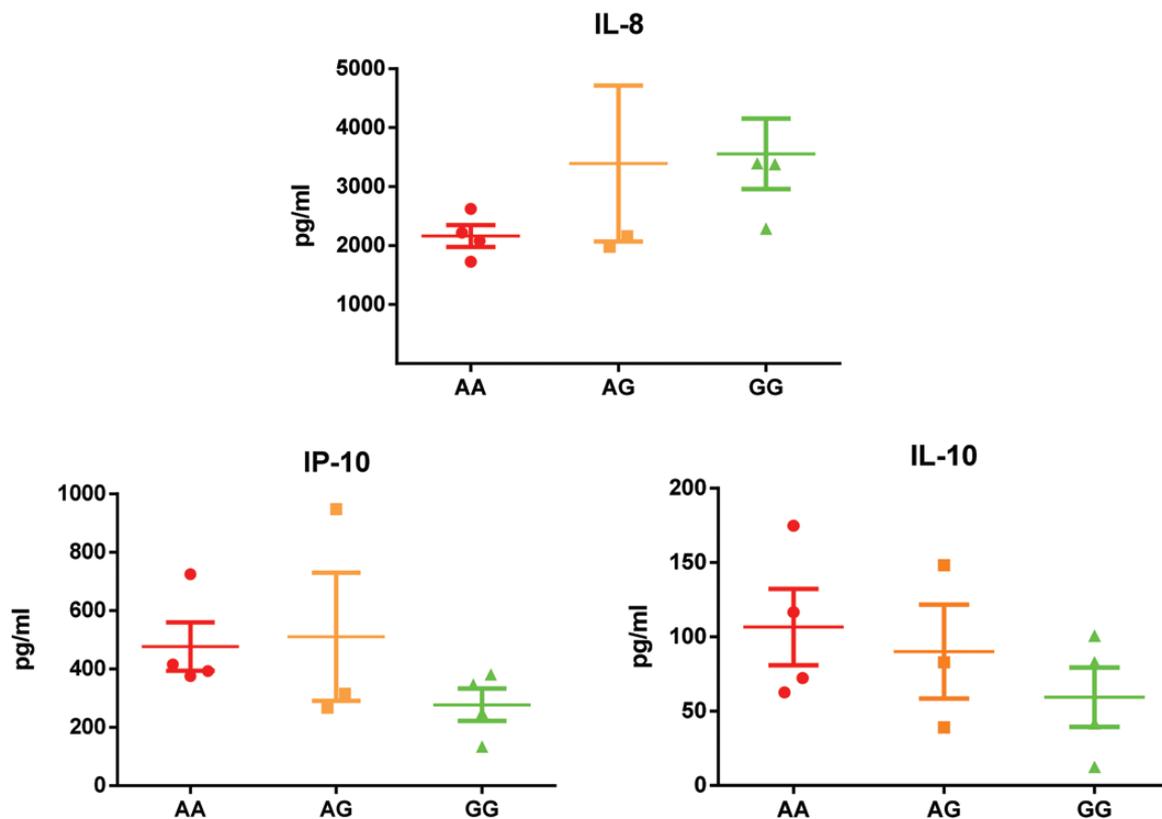
Another important finding of the current study that came from the GWAS in HBeAg-negative patients, were we found *G3BP2* to have the strongest association with both short-term and long-term (Peg)IFN response. This gene has previously been associated with the IFN signaling response as antiviral mediator in the context of viral infection [25]. Therefore, we evaluated whether the SNP could recapitulate the observed association at the functional level. Overnight stimulation of PBMC of healthy controls showed lower protein levels of IL-10 and IP-10, and higher levels of IL-8 with the *G3BP2* response allele G. IL-10 is a well-known immunoregulatory cytokine, which can downregulate T-cell immunity and has a significant role in viral resolution or persistence [26]. Lower levels of IL-10 observed with the *G3BP2* G-allele could therefore be associated with a stronger IFN response. In contrast, earlier studies have shown a negative regulation by IL-8 on the IFN $\alpha$  antiviral response in an HCV system [28, 29], and it is therefore tempting to extrapolate these findings by suggesting that IL-8 may also influence IP-10 levels.

For the complete cohort, we found *rs78900671*, within gene *COL22A1* and close to *TRAPPC9*, to have the strongest association with response. *TRAPPC9*, also known as NIK and IKK $\beta$  binding protein (NIBP), functions as an enhancer of tumor necrosis factor  $\alpha$  induced NF $\kappa$ B activation. NF $\kappa$ B is a multipotent transcription factor, which is involved in many biological processes in (innate) immunity, inflammation and apoptosis [30]. Because it has a broad effect on multiple pathways, studies to decipher the functional consequences of the SNP genotype are difficult to perform and fall outside the scope of the current article. This should be further investigated in future studies.

The current study has been a long-term global investigator-initiated effort in which we have managed to gather the largest (Peg)IFN treated CHB cohort to date. Despite this fact, the Q-Q plots suggest that the study may be underpowered, and the genome-wide significance threshold could not be achieved. Nevertheless, we have found interesting genetic regions for further investigation. In addition, we have performed a power analysis prior to the study and aimed for at least 1000 patients



**Figure 4.** *PRELID2* and response for HBeAg-positive (A) and HBeAg-negative patients (B), and *G3BP2* and response for HBeAg-negative patients (C). Abbreviation: HBeAg, hepatitis B e antigen; LTFU, long-term follow-up.



**Figure 5.** Differential analytes detection for *G3BP2* from supernatants of overnight stimulated PBMC with IFN $\alpha$ +R848 (TLR8 ligand). Abbreviations: IL, interleukin; IP, inducible protein; PBMC, peripheral blood mononuclear cell.

(see [Supplementary Materials](#)). However, because a GWAS is a genetic exploration and the magnitude of minor allele frequencies and the effect of the gene cannot be predicted, we eventually included as many patients as possible.

Given the retrospective nature of our study, inevitably there is a potential of uncontrolled bias due to patient selection. One bias may be to include relatively more nonresponders because patients who achieve a durable response may be discharged and lost to follow-up. This was not the case for the current study. Patients not included in the GWAS ( $N = 637$ ) were more often antiviral therapy experienced, collectively had a lower primary response rate (22%, vs 31% in the GWAS cohort, respectively), and had a similar response rate at end of follow-up of 25%. These percentages are in line with previous (Peg)IFN studies. Another potential caveat could be the heterogeneous population, which could drive the findings in a GWAS if not controlled for. To overcome this potential bias, our GWAS analysis was adjusted for the genetic ancestry principal components of the different populations in the analysis. Moreover, stratified by physician-reported ethnicity the associations remained comparable (also see [Supplementary Materials](#)). This means that the findings from the current study are important for all reported ethnicities. For 30% of patients, we did not have information on

HBsAg levels and for 50% not on HBV genotype, which are both important factors associated with response [23, 31–33]. Indeed, HBV genotype is also strongly related to ethnicity. Because we controlled for the genetic population stratification, chances of spurious genetic associations due to HBV genotypic differences driven by a possible overrepresentation of patients with a certain HBV genotype are highly unlikely but cannot be ruled out.

It is imperative to replicate findings discovered by a GWAS. For the current study we were able to cross-validate SNPs identified by the stratified GWAS. Importantly, here we independently cross-validated *PRELID2*, found in HBeAg-positive patients, for an association with long-term (PEG)IFN response in HBeAg-negative patients. Not unexpectedly, we were not able to replicate *G3BP2* in HBeAg-positive patients, because this was a significant SNP for HBeAg-negative patients. This is a genetically more selected subgroup, and it may not be possible to validate upstream the natural history of CHB. Therefore, we investigated the effect of the SNP on IFN responsiveness in healthy controls. Here we showed that *G3BP2* differentially affects IP-10, IL-10, and IL-8 protein expression. A potential influence of a CHB infection on the effect of the SNP in response to IFN could not be further investigated in the current study. The reason for this is that CHB patients in our clinics are

very heterogeneous in terms of age, phase of infection, antiviral therapy use, and severity of liver disease. Moreover, there is a rather high frequency of Asian patients with a lower frequency of the *G3BP2* AA SNP. All of these factors likely affect the in vitro results and may mask the effect of the SNP on the responsiveness to IFN. Therefore, further studies investigating the function of *G3BP2* should be performed in CHB patients.

In conclusion, we performed to our knowledge the first and largest GWAS study on (Peg)IFN treated CHB patients to date. We found genetic variations associated with response for HBeAg-positive and HBeAg-negative patients, irrespective of ethnicity, both when combined as well as in a stratified analysis, and were able to further independently cross-validate these findings. If these results are further confirmed, this may have important clinical implications for further clinical guidance of patients both in the setting of the natural history, as well as for current or innovative immune modulating therapies.

### Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

### Notes

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version: All authors. W. P. B., A. B., H. L. A. J., and B. E. H. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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