Introduction

Chronic hepatitis B virus (HBV) infection remains a major concern for global health, with an estimated 292 million cases and approximately 30% in China. Because of its high prevalence and severe health consequences, such as cirrhosis and hepatocellular carcinoma (HCC), chronic HBV infection imposes a substantial burden on patients and society. The current first-line antiviral treatments can be classified into two categories: pegylated interferon-alpha (PegIFNα) and nucleos(t)ide analogs (NUCs). NUCs have excellent performance in tolerability, high rates of biochemical remission, and effective inhibition of HBV replication; however, long-term therapies also incur increasing cost,
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safety profiles and compliance issues. As for PegIFNα treatment, despite its severe adverse reactions, it is also an alternative strategy with considerable efficacy in increasing hepatitis B s antigen (HBsAg) loss and reducing the incidence of cirrhosis and HCC.4,5 According to the American Association for the Study of Liver Disease (AASLD) 2018 Hepatitis B Guidance, indicators such as HBV DNA suppression, HBeAg seroconversion or loss, normalization of alanine aminotransferase (ALT), and HBsAg loss, are provided to assess the efficacy of first-line antiviral therapies, including PegIFNα.6 Combined response (CR), defined as HBeAg seroconversion along with HBV DNA level <2000 IU/mL, was also used to evaluate for IFN efficacy.7,8 Regardless of NUCs or PegIFNα, the therapeutic response rate is limited. Therefore, it is important to identify novel biomarkers to predict the therapeutic efficacy.

Accumulating evidence has demonstrated that host genetics could affect the response to IFNα treatment.9,10 Notably, IL28B polymorphism (rs12979860), a predictor of spontaneous clearance of hepatitis C virus (HCV), was strongly associated with therapy response in patients with chronic HCV treated with IFNα and ribavirin.11–13 Genetic markers associated with HBV clearance or hepatitis-related disease progression may also potentially predict drug treatment efficacy. For example, a single-nucleotide polymorphism (SNP) in STAT4 rs7574865 was shown to be associated with the risk of chronic hepatitis B (CHB), HBV-related liver cirrhosis, and HCC in our previous studies.14,15 We further found that rs7574865 was also a potential predictor of treatment response to PegIFNα therapy in hepatitis B e antigen (HBeAg)-positive patients.8,16 Similarly, another SNP, rs12614, which is located in the second exon of CFB and identified as a susceptibility locus for CHB, was also significantly associated with the response to PegIFNα therapy in patients with HBeAg-positive CHB.17,18

Granulysin (GNLY) is a saposin-like pore-forming protein that acts as a cytotoxic granule and is mainly secreted by cytotoxic T lymphocytes and natural killer (NK) cells in mammals. It exerts a toxic role with respect to bacteria, fungi, parasites, and tumors,19,20 and acts as an anti-virus biomarker, such as in Epstein-Barr virus (EBV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.21,22 Encoded by the GNLY gene in chromosome 2p11.2, GNLY has two isoforms, unprocessed 15 kDa and the processed 9 kDa. The recombinant 9-kDa protein has been demonstrated its broadly cytotoxic and antimicrobial properties, while 15 kDa isoform may function as an immune “alarmin”, promoting the maturation and migration of antigen-presenting cells and other immune cells.23 It has been reported that GNLY exerts antiviral effects by inhibiting the replication of varicella-zoster virus and triggering apoptosis in infected cells.24 However, correlations between GNLY and hepatitis viruses have been limited to association studies, and specific anti-virus mechanistic studies have not been reported. A common missense polymorphism of GNLY, rs11127, was previously reported to be significantly associated with HBV clearance in patients with CHB.25,26 However, whether the different genotypes of GNLY rs11127 influence the treatment efficacy of PegIFNα or NUCs remains to be elucidated.

This study first evaluated the predictive performance of GNLY rs11127 in response to CHB therapies in two PegIFNα and two NUCs cohorts. We then compared GNLY expression levels in patients with different GNLY rs11127 genotypes using data from the Genotype-Tissue Expression Project (GTEx). Finally, a polygenic score (PGS) integrating GNLY rs11127 and three other SNPs (STAT4 rs7574865, CFB rs12614, and CD55 rs28371597),8,18 which were associated with PegIFNα treatment response, was established to predict the possibility of PegIFNα therapy response.

**Patients and Methods**

**Patients**

We included a total of 1823 CHB patients from four Phase IV, multicenter, randomized controlled trials, and they were retrospectively analyzed. Patients in two trials (PegIFNα cohort 1, n = 246 and PegIFNα cohort 2, n = 708) were treated with PegIFNα-2a/2b for at least 48 weeks, and those in the other two trials (NUCs cohort 1, n = 553, and NUCs cohort 2, n = 316) were treated with NUCs for 104 weeks. Details of patient allocation and specific treatment regimens for each cohort are described in Supplementary Table 1, and the inclusion and exclusion criteria of the patients were previously described.27–30 Briefly, eligible patients were aged 18–65 years, with positive HBsAg for at least 6 months, positive HBeAg, HBV DNA >1.7×10⁴ IU/mL (10⁵ copies/mL) or 2×10⁴ IU/mL (PegIFNα cohort 2), and ALT ≥2 (except ALT ≥1.3 in NUCs cohort 2) and <10 × upper limits of normal (ULN). We excluded patients who had received antiviral treatment within the prior 6 months or were infected with other chronic liver diseases, including decompensated liver disease and HCC. The study flowchart is provided in Figure 1.
This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Nanfang Hospital (PegIFNα cohort 1 – NCT01086085, PegIFNα cohort 2 – NCT01760122, NUCs cohort 1 – NCT00962533, NUCs cohort 2 – NCT01088009). Furthermore, written informed consent was obtained from all participants.

**DNA Extraction and Genotyping of Genetic Polymorphisms**

Genomic DNA was extracted from the peripheral blood of patients with CHB using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Genotyping of rs11127 was performed using the Sequenom MassARRAY platform (Sequenom, San Diego, CA, USA) according to the instructions at the Fudan-VARI Center of Genetic Epidemiology, Fudan University. The polymerase chain reaction was conducted using 10ng of genomic DNA in 5μL volumes. Twenty duplicate test samples were genotyped in a double-blind manner. The genotyping concordance was 100% for duplicate samples.

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**Figure 1** Flowchart of the study procedure.

**Abbreviations:** HBV, hepatitis B virus; PegIFNα, pegylated interferon alpha; NUCs, nucleoside analogues; CR, combined response; GNLY, granulysin; HBeAg, hepatitis B e antigen; SNPs, single-nucleotide polymorphisms; PGS, polygenic score.
Serologic Testing and Efficacy Evaluation

Clinical laboratory parameters were evaluated every 8–16 weeks during the trial. HBV DNA levels were detected using the Roche COBAS TaqMan platform (Hoffmann-La Roche Ltd, Basel, Switzerland), with a lower limit of detection (LLOD) of 12 IU/mL or 69.84 copies/mL. HBV serological markers were measured using ARCHITECT i2000SR (Abbott Laboratories, Chicago, IL, USA) at the central laboratory of each research group. Results below the detection limit were substituted by LLOD.

The primary efficacy endpoints were CR and HBsAg loss in this study. CR was defined as HBeAg seroconversion (loss of HBeAg and the presence of HBeAb) along with an HBV DNA level <2000 IU/mL at week 72 in PegIFNα cohorts or week 104 in NUCs cohorts. HBsAg loss was defined as an HBsAg titer lower than the detection limit (HBsAg <0.05 IU/mL) at the corresponding endpoints. Patients without HBeAg seroconversion and with an HBV DNA level ≥2000 IU/mL at the corresponding endpoints were classified as non-responders.

Analysis of GNLY Expression Levels in Whole Blood and Liver Tissues with Different rs11127 Alleles

To further investigate the potential association of the rs11127 genotypes with GNLY expression level, the expression of GNLY in liver tissue or blood and the rs11127 genotype information were downloaded from the GTEx portal website (https://gtexportal.org/home/testyourown). Violin plot was used to visualize GNLY expression levels according to different rs11127 genotypes.

Statistical Analyses

Continuous variables were presented as mean ± standard deviation (SD), and categorical variables were presented as counts (percentages). The Hardy-Weinberg equilibrium (HWE) of the rs11127 genotype distribution in CHB patients was assessed using a chi-square test. The association of rs11127 with CR was analyzed using the Cochran-Armitage trend test. Multiple logistic regression analysis was performed using a backward elimination process, in which only factors with a P < 0.25 in the univariate logistic regression analysis were included. In addition, the factors with a P-value < 0.1 in the backward selection procedure remained in the model.

Results

Clinical Characteristics

The demographics, HBV genotypes, and HBsAg levels were generally similar between the PegIFNα and NUCs cohorts, as shown in Table 1. Genotyping of rs11127 failed in 17 patients (1, 12, 4, and 1, in PegIFNα cohort 1/2 and NUCs cohort 1/2, respectively). Patients were predominantly men and the majority of patients were from Han Chinese ancestry (96.44% in the PegIFNα cohort and 96.78% in the NUCs cohort). Only Han Chinese patients were included for further analyses to minimize the influence of genetic heterogeneity. In terms of HBV genotypes, C was predominant (58.70–61.45%), followed by B (37.97–38.26%). HBsAg levels in terms of HBV genotypes, C was predominant (58.70–61.45%), followed by B (37.97–38.26%). HBsAg levels were comparable between the two combined cohorts. Other HBV serological markers at baseline levels, such as HBV DNA and HBeAg, were higher in the PegIFNα than the NUCs cohorts (Table 1). The level of ALT was 4.47 × ULN in the combination of the two PegIFNα cohorts and 3.98 × ULN in the combination of the two NUCs cohorts, respectively. In addition, the proportions of GNLY rs11127 (CC, CT, and TT) were very similar in patients treated with PegIFNα and those treated with NUCs (P = 0.635), and no obvious correlation with other baseline variables was found in PegIFNα-treated patients and NUCs-treated patients (Supplementary Table 2).
Association of GNLY rs11127 with CR in the PegIFNα and NUCs Cohorts

Allele distributions of GNLY rs11127 fulfilled HWE in four cohorts and minor allele frequency (MAF) was >0.4 in each cohort. Based on predefined inclusion and exclusion criteria, 179 (responders and non-responders = 65/114), 436 (128/308), 199 (135/64) and 129 (53/76) patients remained in PegIFNα cohort 1/2 and NUCs cohort 1/2, respectively.

As shown in Figure 2, 28.57%, 38.30%, and 44.83% of patients with rs11127 TT, CT, and CC genotypes in PegIFNα cohort 1 achieved CR, respectively. In addition, 26.53%, 26.53%, and 39.78% in PegIFNα cohort 2 achieved CR. When combining the two PegIFNα cohorts, we found that the CR rates in patients with the rs11127 TT, CT, and CC genotypes were 27.09%, 30.34%, and 40.98%, respectively. Univariate logistic regression analysis found significant correlations between GNLY rs11127 and CR were also remained in the PegIFNα cohort 2 (OR = 1.55, P = 0.008) and the combined PegIFNα cohort (OR = 1.56, P = 0.003, Figure 2A–C and Table 2). Further, by including clinical variables and SNPs, we constructed a regression model for predicting CR, which was as follow: log(P/(1-P)) = −0.005–0.060* Age + 0.685 * HBV genotype − 1.143 * HBeAg + 0.902 * ALT + 1.137 * CFB genotype + 1.503 * CD55 genotype + 0.443 * GNLY genotype. However, no statistically significant association between rs11127 and CR rate was found in the two NUCs cohorts (Supplementary Figure 1).

Expression of GNLY in Whole Blood and Liver Tissues Between Different rs11127 Alleles

GTEx data has been representing the largest atlas of the catalog of trait loci and human gene expression. We assessed GNLY expression levels in whole blood and liver tissues with different rs11127 genotypes using the GTEx website to further investigate the effect of the rs11127 genotype on the mRNA expression of GNLY. Different GNLY expression

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**Table 1** Characteristics of Patients in PegIFNα Cohorts and NUCs Cohorts

| Clinical Variables | PegIFNα Cohorts | NUCs Cohorts | P-value (PegIFNα Cohort Combination vs NUCs Cohort Combination) |
|--------------------|-----------------|--------------|---------------------------------------------------------------|
|                    | PegIFNα Cohort 1 (n = 246) | PegIFNα Cohort 2 (n = 708) | PegIFNα Cohort Combination (n = 954) | NUCs Cohort 1 (n = 553) | NUCs Cohort 2 (n = 316) | NUCs Cohort Combination (n = 869) |
| Male gender (%)    | 194 (78.90) | 512 (72.30) | 706 (74.00) | 450 (81.40) | 239 (75.60) | 689 (79.29) | 0.008 |
| Age, years; mean (SD) | 29.05 (6.79) | 29.743 (6.70) | 29.56 (6.73) | 30.13 (8.96) | 31.94 (9.40) | 30.79 (9.16) | 0.001 |
| Han ethnicity (%)  | 243 (98.80) | 677 (95.60) | 920 (96.44) | 540 (97.70) | 301 (95.30) | 841 (96.78) | 0.629 |

**Abbreviations:** PegIFNα, pegylated interferon alpha; NUCs, nucleoside analogues; SD, standard deviation; HBV, hepatitis B virus; NA, not available; HBeAg, hepatitis B surface antigen; HBeAg*, log10 IU/mL; mean (SD); HBsAg*, log10 IU/mL; mean (SD); HBV DNA*, log10 IU/mL; mean (SD); ALT*, ×ULN; mean (SD); GNLY, granulysin.

**Notes:** aBaseline level. The P values of each baseline variable were calculated by comparing PegIFNα cohort combination and NUCs cohort combination.

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**Table 2** Liver Tissues Between Different rs11127 Alleles

| Alleles | Liver Tissues |
|---------|--------------|
| CC      | Liver Tissues |
| CT      | Liver Tissues |
| TT      | Liver Tissues |
| NA      | Liver Tissues |

**Abbreviations:** GNLY, granulysin; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; ULN, upper limit of normal; GNLY, granulysin.
levels were found among individuals with different genotypes of the genetic variant. As shown in Figure 3, compared with those carrying rs11127 CT or TT, individuals carrying rs11127 CC showed higher GNLY expression levels in whole blood \( (P = 1.700 \times 10^{-27}) \) or liver tissues \( (P = 0.0001) \).

**Association of PGS with CR in the PegIFNα Cohorts**

In our previous studies, \( STAT4 \) rs7574865, \( CFB \) rs12614, and \( CD55 \) rs28371597 were identified as reliable predictors of treatment response in patients with HBeAg-positive CHB patients treated with PegIFNα.\(^8,^{18} \) A PGS was constructed to assess the cumulative effect of the SNPs and \( GNLY \) rs11127. For each SNP, homozygous non-favorable alleles, heterozygous, and homozygous for favorable alleles were coded as 0, 1, and 2, respectively. The PGS was calculated for each participant as the sum of the favorable alleles.

We have found that the CR rate steadily increased with an increase in PGS (Figure 4A–C). In all patients treated with PegIFNα, the CR rates in patients carrying five and six favorable alleles were 48.89%, and 50%, respectively. These CR rates were higher than the proportions of 23.4%, 25.87%, and 32.1% in patients carrying two, three, and four favorable alleles, respectively. After adjusting for other baseline variables, the association between PGS and CR was significant in PegIFNα cohort 1 \( (OR = 1.87, \ P = 0.004) \), PegIFNα cohort 2 \( (OR = 1.44, \ P = 0.001) \), and the combined PegIFNα cohort \( (OR = 1.52, \ P < 0.001, \ Table \ 3) \).

In addition, we constructed regression models for predicting PegIFNα efficacy by including clinical variables combined with PGS: model 2, or including clinical variables only: model 1. Through further ROC contrast estimation and testing, the results demonstrated that the performance of the model including clinical variables combined with PGS in predicting PegIFNα efficacy was remarkably improved compared to that of the model including clinical variables only \( (P = 0.025, \ Supplementary \ Table \ 4) \).

**Discussion**

It is important to predict the treatment efficiency to target CHB patients, as PEG-IFN is used as a first-line antiviral therapy in adults and the treatment response rate is limited. Previous studies suggest that response-guided therapy or early on-treatment HBV RNA levels could be of great help in the management of patients treated with PegIFNα,\(^{29,31} \) while it is unclear which indicators can predict that patients with CHB could benefit from earlier treatment. Although several SNPs have been proposed,\(^8,^{32,33} \) the effect of \( GNLY \) rs11127 on PegIFNα efficacy has never been reported before. Previously, \( GNLY \) rs11127 was regarded as an important factor in HBV clearance in patients infected with HBV in a Korean study.\(^{25} \) The present study systematically evaluated the performance of \( GNLY \) rs11127 in predicting the response to PegIFNα or NUCs in Chinese HBeAg-positive CHB patients from four multicenter, randomized controlled trials. The missense variant rs11127 was significantly associated with CR in PegIFNα-treated patients, but not in those treated with NUCs. In addition, we constructed a robust PGS model including \( STAT4 \) rs7574865, \( CFB \) rs12614, \( CD55 \) rs28371597 and \( GNLY \) rs11127, which significantly improved the prediction of PegIFNα treatment efficacy.
Table 2 Multivariate Logistic Regression Analysis of GNLY Rs11127 with CR in PegIFNα Cohorts

| Parameters                        | PegIFNα Cohort 1 |          | PegIFNα Cohort 2 |          | PegIFNα Cohort Combination |          |
|----------------------------------|------------------|----------|------------------|----------|---------------------------|----------|
|                                  | Beta             | OR (95% CI) | P                | Beta     | OR (95% CI) | P       | Beta     | OR (95% CI) | P       |
| Intercept                        | -0.491           |          | 0.464            | 4.451    |          | <0.001 | -0.005   |          | 0.997   |
| Trial (PegIFNα cohort 1 vs 2)    | -                |          |                  |          | -        |        |          | -        |        |
| Gender (Female)                  | -                |          |                  | -        | -        |        | -        | -        |        |
| Age, years                       | -                |          |                  | -        | -        |        | -        | -        |        |
| HBV genotype (B vs C)            | 0.778            | 2.18 (0.91–5.20) | 0.080 | 0.697 | 2.01 (1.25–3.21) | 0.004 | 0.685 | 1.98 (1.32–2.99) | 0.001 |
| HBV DNA*, log10 IU/mL*           | -                |          |                  | -        | -        |        | -        | -        |        |
| HBeAg*, log10 PEIU/mL            | -                |          |                  | -        | -        |        | -        | -        |        |
| ALT*, loge × ULN                 | 1.024            | 3.61 (1.82–7.16) | <0.001 | 1.599 | 1.75 (1.03–2.98) | 0.039 | 0.902 | 2.47 (1.66–3.67) | <0.001 |
| STAT4 genotype                   | 0.531            | 1.70 (0.95–3.04) | 0.074 | -        |          |        | -        |          |        |
| CFB genotype                     | -                |          |                  | -        | -        |        | -        |          |        |
| CD55 genotype                    | 1.505            | 4.50 (1.02–19.92) | 0.047 | 1.646 | 5.19 (2.25–11.98) | <0.001 | 1.503 | 4.49 (2.16–9.35) | <0.001 |
| GNLY genotype                    | -                |          |                  | -        | -        |        | -        |          |        |

Note: *Baseline level.

Abbreviations: OR, combined response; PegIFNα, pegylated interferon alpha; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; OR, odds ratio; CI, confidence interval; ALT, alanine aminotransferase; ULN, upper limit of normal; STAT4, signal transducer and activator of transcription 4; CFB, complement factor B; GNLY, granulysin.

Currently, predictive models of the efficacy for initial treatment with PegIFNα were mainly reported on studies of some clinical baseline indicators or SNPs. However, none of the published literature states if SNPs could improve the performance of clinical variables combined with PegIFNα, only in predicting PegIFNα efficacy. The results demonstrated that SNPs combined with PegIFNα should be used to predict the efficacy of PegIFNα, and the model, including clinical variables only, may also provide a more practical reference for precise treatment strategies for HBeAg-positive CHB patients.

Therefore, we compared the performance of the models including clinical variables combined with PegIFNα with that of the model only on clinical variables and the model including only clinical variables.
granule into the infected cells, and then granulysin delivers granzymes into bacteria to kill diverse bacteria.\(^\text{41}\) However, GNLY is usually reported as a cytolytic effector in viral diseases, such as HBV and SARS-CoV-2 infection, and its antiviral mechanism is still unclear.\(^\text{21}\) The rs11127 SNP, located in the 4th exon of GNLY, changes the amino acid, which may influence the treatment efficacy of PegIFNα. Furthermore, data from GTEx revealed that the GNLY mRNA expression levels varied depending on the rs11127 genotypes, which may be caused by the change in mRNA stability or potential linkage disequilibrium between the SNP and some functional sites in the regulatory region.

A previous study on the association of GNLY genetic polymorphisms with CHB infection in Korea showed that the T allele-containing genotype of rs11127 was associated with HBV clearance.\(^\text{25}\) However, in this study, we found that the C allele of rs11127 was the favorable allele with a higher CR in patients with CHB treated with PegIFNα. Our results were consistent with those of a Chinese study, which showed significantly reduced susceptibility to HBV infection in men carrying the rs11127 TC genotype and women carrying the rs11127 CC genotype.\(^\text{26}\) However, the underlying mechanism of these discrepant results remains unclear. A possible explanation is the different ethnic and hereditary backgrounds of the Korean and Chinese patients.

With the continuous decrease in the cost of genotyping chips, PGS has great potential for the precise prediction of diseases or treatment efficacy. Gellert-Kristensen found a genetic risk score including three common variants conferred
up to a 12-fold higher risk of cirrhosis, and a 29-fold higher risk of HCC in patients with fatty liver disease.\textsuperscript{42} Thus, this study, we constructed a PGS model to evaluate the cumulative effect of GNLY rs11127, \textit{STAT4} rs7574865, CFB rs12614, and \textit{CD55} rs28371597, all of which were previously identified as predictors of treatment response to PegIFN\(\alpha\) therapy. We found that the CR rate increased steadily with an increase in PGS in the PegIFN\(\alpha\) cohorts. The CR rate in patients with a PGS of six was approximately 1-fold higher than that in patients with a PGS of 2 or 3. No individuals with a PGS equal to one achieved a treatment response to PegIFN\(\alpha\) therapy, and nearly half of the patients could benefit from PegIFN\(\alpha\) therapy if PGS $\geq 5$. The PGS has implications for the clinical management and selection of PegIFN\(\alpha\) therapy in HBeAg-positive CHB patients. We expect PGS to comprise more IFN\(\alpha\) treatment response-related SNPs, which would be more effective in predicting the treatment response to PegIFN\(\alpha\).

Compared with NUCs, PegIFN\(\alpha\) was reported to have a greater chance of achieving HBeAg seroconversion and HBSAg clearance, which are associated with improved clinical outcomes. However, no significant association of rs11127 with HBSAg loss was found in the PegIFN\(\alpha\) cohorts (Supplementary Figure 2) because of the limited sample size which attenuated the statistical power. Therefore, further studies with larger sample sizes are needed to validate the association between rs11127 and HBSAg loss in the future.

## Conclusions
\textit{GNLY} rs11127 was significantly associated with treatment response to PegIFN\(\alpha\) therapy in Chinese patients with HBeAg-positive CHB. A PGS comprising \textit{GNLY}, \textit{STAT4}, \textit{CFB}, and \textit{CD55} variants could effectively predict the CR rate in PegIFN\(\alpha\)-treated CHB patients, which may have implications for clinical management and individualized treatment guidance.

## Abbreviations
ALT, alanine aminotransferase; CHB, chronic hepatitis B; CR, combined response; GNLY, Granulysin; GTEx, Genotype-Tissue Expression Project; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; NK, natural killer; NUCs, nucleos(t)ide analogs; PegIFN\(\alpha\), pegylated-interferon-alpha; PGS, polygenic score; ROC, receiver operating characteristic curve; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; SNPs, single-nucleotide polymorphisms; ULN, upper limit of normal.

## Data Sharing Statement
All data generated or analysed during this study are included in this published article and its additional files. The public data during the current study are available in the GTEx (https://gtexportal.org/home/testyourown).

## Ethics Approval and Informed Consent
This study was approved by the Ethics Committee of Nanfang Hospital. Written informed consent was obtained from all patients or their family members. The data from GTEx are shared and available to the public, and there are no ethical concerns. The study was performed and reported in accordance with the Helsinki Declaration.

## Table 3 Multivariate Logistic Regression Analysis of PGS with CR in PegIFN\(\alpha\) Cohorts

| Parameters | PegIFN\(\alpha\) Cohort 1 | PegIFN\(\alpha\) Cohort 2 | PegIFN\(\alpha\) Cohort Combination |
|------------|--------------------------|--------------------------|-----------------------------------|
| OR (95% CI) | P | OR (95% CI) | P | OR (95% CI) | P |
| Trial (PegIFN\(\alpha\) cohort 1 vs 2) | – | 0.79 (0.48–1.32) | 0.376 | – | – |
| Gender (Female) | 0.87 (0.31–2.45) | 0.788 | 1.38 (0.82–2.33) | 0.223 | 1.29 (0.82–2.05) | 0.277 |
| Age, years | 0.96 (0.90–1.03) | 0.228 | 0.93 (0.90–0.97) | < 0.001 | 0.94 (0.91–0.97) | < 0.001 |
| HBV genotype (B vs C) | 2.58 (1.00–6.68) | 0.050 | 1.99 (1.23–3.21) | 0.005 | 2.06 (1.35–3.14) | < 0.001 |
| HBV DNA\textsuperscript{*}, log\textsubscript{10} IU/mL | 1.33 (0.75–2.38) | 0.328 | 0.97 (0.63–1.47) | 0.869 | 1.16 (0.84–1.6) | 0.377 |
| HBsAg\textsuperscript{*}, log\textsubscript{10} IU/mL | 0.57 (0.24–1.33) | 0.192 | 0.97 (0.53–1.76) | 0.913 | 0.79 (0.49–1.26) | 0.318 |
| HBeAg\textsuperscript{*}, log\textsubscript{10} PEIU/mL | 0.34 (0.17–0.66) | 0.001 | 0.25 (0.14–0.43) | < 0.001 | 0.31 (0.21–0.46) | < 0.001 |
| ALT\textsuperscript{*}, log\textsubscript{10} ×ULN | 3.64 (1.77–7.48) | < 0.001 | 1.86 (1.09–3.17) | 0.023 | 2.53 (1.69–3.79) | < 0.001 |
| PGS | 1.87 (1.23–2.85) | 0.004 | 1.44 (1.15–1.80) | 0.001 | 1.52 (1.25–1.85) | < 0.001 |

Note: \textsuperscript{*}Baseline level.

Abbreviations: CR, combined response; PegIFN\(\alpha\), pegylated interferon alpha; HBV, hepatitis B virus; HBSAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; OR, odds ratio; CI, confidence interval; ALT, alanine aminotransferase; ULN, upper limit of normal; PGS, polygenic score.
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Disclosure

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