



Original Article



CD55 Variant Associated with Pegylated-interferon α Therapy Response in HBeAg-positive Chronic Hepatitis B Patients

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Abstract

Background and Aims: Only a small percentage of chronic hepatitis B (CHB) patients effectively respond to treatment with pegylated-interferon alpha (PegIFN α) or nucleos(t)ide analogues (NUCs). We aimed to detect the correlations of complement regulators-associated single-nucleotide polymorphisms (SNPs) with treatment response of hepatitis B e antigen (HBeAg)-positive CHB patients. **Methods:** A total of 1,763 HBeAg-positive CHB patients were enrolled, 894 received PegIFN α for at least 48 weeks and were followed up for 24 weeks, and 869 received NUCs for 104 weeks. For each patient, nine SNPs in genes encoding for complement regulators were determined and genotyped. To assess the cumulative effect of numerous SNPs, a polygenic score (PGS) was utilized. The correlations of SNPs and PGS with the levels of combined response (CR) and hepatitis B s antigen (HBsAg) loss were also investigated. **Results:** In PegIFN α -treated patients, an intronic SNP of *CD55*, rs28371597, was strongly related to CR, and the CR rate in rs28371597_GG genotype carriers was only approximately half that of rs28371597_GT/TT genotype carriers (20.29% vs. 37.10%, $p=2.00 \times 10^{-3}$). A PGS incorporating *CD55*, rs28371597 and two additional SNPs, *CFB*_rs12614 and *STAT4*_rs7574865, which had been considered as predictors for PegIFN α treatment response before, was strongly correlated with the levels of CR (p -trend=7.94 $\times 10^{-6}$) and HBsAg loss (p -trend=9.40 $\times 10^{-3}$) in PegIFN α -treated patients. In NUCs-treated individuals, how-

ever, none of the nine SNPs were shown to be significantly linked to CHB treatment response. **Conclusions:** *CD55*_rs28371597 is a promising biomarker for predicting CHB patients' responsiveness to PegIFN α therapy. The updated PGS may be used for optimizing CHB treatment.

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Introduction

Hepatitis B virus (HBV) infection is a serious health problem worldwide. More than 250 million people are chronic hepatitis B (CHB) patients with the potential to cause persistent infection and eventually develop into severe outcome such as liver failure and hepatocellular carcinoma (HCC).¹ Interferon alpha (IFN α) or pegylated-interferon alpha (PegIFN α) performs as immune modulators, and nucleos(t)ide analogues (NUCs) with an antiviral role, are currently the main antiviral treatments of HBV.² PegIFN α can achieve sustained off-treatment control, although it benefits only a fraction of CHB patients and has undesirable side effects.³ NUCs are efficient at controlling HBV replication, meanwhile they should be taken on a long-term for the majority of CHB patients.⁴ However, hepatitis B surface antigen (HBsAg) seroclearance can be observed in only a tiny portion of CHB patients following long-term treatment.⁴ Therefore, it is imperative to identify determinants of CHB treatment outcomes to improve personalized therapy. According to several publications, numerous host-related and virus-related factors with an impact on therapeutic efficiency that were deemed outcome predictors for treatment response were ineffective at predicting therapy response at the customized level.⁵⁻⁷

A growing body of research suggests that host genetics may influence CHB patients' responsiveness to IFN α treatment.⁸⁻¹⁰ Our 2016 study demonstrated that *STAT4*_rs7574865 was significantly correlated with PegIFN α therapy response in 466 hepatitis B e antigen (HBeAg)-positive CHB patients.⁸ Moreover, *STAT4*_rs7574865 was recently proven to be related to PegIFN α treatment outcomes in CHB

Keywords: Complement regulator; Chronic hepatitis B patients; Polygenic score; Single-nucleotide polymorphism; Treatment response.

Abbreviations: ALT, alanine aminotransferase; APC, antigen-presenting cell; ADV, adenofovir; cccDNA, covalently closed circular DNA; C1INH, C1 inhibitor; C4BP, C4b binding protein; CFB, complement factor B; CFD, complement factor D; CFH, complement factor H; CFI, complement factor I; CHB, chronic hepatitis B; CR, combined response; DAF, decay accelerating factor; eQTL, expression quantitative trait locus; GPI, glycosylphosphatidylinositol; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IFN α , interferon alpha; LAM, lamivudine; LD, linkage disequilibrium; LOD, lower limit of detection; NK, natural killer; NUC, nucleos(t)ide analog; PegIFN α , pegylated-interferon alpha; PGS, polygenic score; SD, standard deviation; SNP, single-nucleotide polymorphism; TRAIL, TNF-related apoptosis-inducing ligand; ULN, upper limit of normal.

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patients.⁹ In 2020, we discovered another single-nucleotide polymorphism (SNP) named *CFB*_rs12614, which is a susceptibility locus for CHB,¹¹ also had a close association with PegIFN α treatment response.¹⁰

As a complement system regulator, *CFB* plays a vital function in enhancing the complement response and effectively eliminating pathogens.¹² Inspired by the study of *CFB*_rs12614, other potentially functional SNPs in the genes encoding complement regulatory proteins should be investigated to see whether they might predict CHB patients' abilities in PegIFN α or NUCs treatment response.

Complement regulatory soluble proteins such as C1 inhibitor (C1INH), C4b binding protein (C4BP) and complement factors B, D, H and I (*CFB*, *CFD*, *CFH* and *CFI*), are more specific, and control either the alternative or the classical or the lectin pathways. Complement regulatory cell-membrane-anchored proteins such as CD35, CD46, CD55, and CD59, control the three complement activation pathways.¹³

In this study, we comprehensively assessed the efficacy of complement regulators-associated SNPs in predicting the clinical outcomes of CHB treatments by analyzing four trials including patients received PegIFN α treatment or NUCs treatment.

Methods

Patients

A retrospective cohort analysis included 1,763 participants who were recruited in four trials that consisted of EXCEL-PegIFN α cohort 1 ($n=186$), PB-PegIFN α cohort 2 ($n=708$), EFFORT-NUCs cohort 1 ($n=553$) and EXPLORE-NUCs cohort 2 ($n=316$). Eligible patients (18 to 65 years of age) enrolled in all four trials had similar inclusion and exclusion criteria, including HBsAg positive for at least 6 months, HBeAg positive, HBV DNA $>10^5$ copies/mL, alanine aminotransferase (ALT) $\geq 2 \times$ the upper limit of normal (ULN) but $<10 \times$ ULN and without antiviral treatment within 6 months. Other major exclusion criteria included coinfection with hepatitis C, hepatitis D, or the human immunodeficiency virus, presence of chronic liver disease other than viral hepatitis, evidence of hepatic decompensation, portal hypertension, or HCC.

In EXCEL-PegIFN α cohort 1, patients were enrolled across 11 clinical centers in China between April 2010 and October 2010, and received PegIFN α (180 μ g/week) for 24 weeks prior to randomization. The levels of serum HBV DNA and HBsAg were detected at week 24. Patients with HBsAg <500 IU/mL and HBV DNA $<10^5$ copies/mL were defined as early responders, while others were defined as non-early responders.¹⁴ Early responders were treated with PegIFN α for a further 24 weeks (total 48 weeks). Non-early responders continued for another 24 or 72 weeks (total 48 or 96 weeks). PB-PegIFN α cohort 2 involved 43 study locations lasting from January 2013 to August 2015. Eligible patients were randomized to PegIFN α -2b group or PegIFN α -2a group.¹⁵ All patients were treated with PegIFN α -2b or PegIFN α -2a at 180 μ g/week for 48 weeks and were followed up for 24 weeks. EFFORT-NUCs cohort 1 was conducted at 24 centers in China from August 2009 to March 2012. Patients with suboptimal response (24-week \geq HBV DNA 300 copies/mL) started telbivudine 600 mg daily and added adefovir (ADV) 10 mg daily from week 28 to week 104, while others received telbivudine monotherapy for 104 weeks.¹⁶ Another NUCs cohort was a randomized, and controlled study in 24 centers of China from March 2010 to February 2013. Patients were treated with lamivudine (LAM) for 24 weeks, after which those suboptimal responders with HBV DNA $>1,000$ copies/mL at week 24 received LAM plus adefovir until week 104, while the early virological responders continued LAM monotherapy.¹⁷

In addition, some patients received LAM plus ADV combination therapy for 104 weeks.¹⁷ Detailed patient allocation and treatment regimens for each trial have already been described previously.^{14–17} Additionally, each trial's demographic, virologic, and clinical parameters have been reported in previous.⁹ The study protocol followed the 2013 revision of the guidelines of the Declaration of Helsinki and the Nanfang Hospital Ethics Committee authorized this study. All patients signed an informed consent form.

Serological testing

Throughout the period of four trials, clinical, and laboratory evaluations were done every 8–16 weeks. The Roche COBAS Taqman platform and ARCHITECT i2000SR (Abbott Laboratories, Chicago, IL, USA) were utilized to detect the levels of HBV DNA and other virological indicators. The results were replaced with a lower limit of detection (LLOD) of 12 IU/mL or 69.84 copies/mL when they were lower than the LLOD.

Efficacy measures

The combined response (CR) rate and the level of HBsAg loss were considered as endpoint responses to assess therapy efficacy. The combination of HBeAg seroconversion (removal of HBeAg and the presence of HBeAg antibody) and reduced HBV DNA level (HBV DNA $<2,000$ IU/mL at week 72 for the PegIFN α cohorts or HBV DNA <12 IU/mL at week 104 for the NUCs cohorts) was classified as CR. The level of HBsAg in serum less than 0.05 IU/mL at week 72 in the PegIFN α cohorts or at week 104 in the NUCs cohorts was regarded as HBsAg loss. The detailed information was described previously.^{14–17}

SNP selection and genotyping

The complement regulators-associated SNPs were selected through a screening process shown in Supplementary Figure 1. Briefly, the genes coding complement regulatory proteins were annotated by GENCODE, Build 37 (Supplementary Table 1). Based on the 1000 Genomes Project database, 512 SNPs located in the genes coding complement regulatory proteins were included after quality control (minor allele frequency >0.05 , missing rate <0.05 , p -value of Hardy-Weinberg equilibrium >0.05). To focus on potentially functional SNPs that may affect gene expression, an expression quantitative trait locus (eQTL) analysis in whole blood was conducted (SNPs with $p < 0.01$ were considered significant), and 103 SNPs were remained (Supplementary Table 2). As some of the 103 SNPs are in high linkage disequilibrium (LD) with each other, a clump analysis was performed to screen out independent SNPs. Nine independent SNPs were chosen for further investigation (Supplementary Table 3). Peripheral blood was used to extract genomic DNA with a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. Genotyping of the nine selected SNPs in genomic DNA samples of all the CHB patients was analyzed by the Sequenom mass array platform (Sequenom, San Diego, CA, USA).

Statistical analysis

The chi-squared test or Fisher exact test for categorical parameters and the Student t -test or the Mann-Whitney test for continuous parameters were used to evaluate qualita-

tive and quantitative differences across subgroups, as appropriate. By computing the frequency of favorable alleles, a polygenic score (PGS) was established to measure the cumulative effects of several SNPs recognized as indicators of CHB treatment response. Patients who possessed more favorable alleles had a higher level of CR or HBsAg loss. The Cochran-Armitage trend test was performed to analyze the correlations of PGS with CR level and HBsAg loss. All statistical tests were two sided. Two-tailed p -values <0.05 were considered statistically significant. SAS 9.3 (SAS Institute, Cary, NC, USA) was used to perform the statistical analysis.

Results

Patient characteristics

The correlations of complement regulators-associated SNPs with CHB therapies were evaluated in two PegIFNa cohorts with total 894 CHB patients and two NUCs cohorts with total 869 CHB patients. Patients in the PegIFNa and NUC cohorts were comparable in baseline demographic, virological, and clinical characteristics, as shown in Table 1. Male patients accounted for 74.16% of patients received PegIFNa treatment and 79.29% of patients received NUCs treatment. The majority of patients were Han Chinese (94.18% in PegIFNa-treated patients and 96.78% in NUCs-treated patients) and only Han Chinese were involved in the subsequent analyses for reducing genetic heterogeneity. Most of the CHB patients infected with genotype B or C HBV, which took over approximately 98.00% of all the CHB patients. The baseline levels of HBV serological markers in PegIFNa-treated patients were almost the same as the NUCs-treated patients. Moreover, the level of ALT was higher in PegIFNa-treated patients than in NUCs-treated patients.

Additionally, as mentioned earlier, our group indicated a statistically significant connection between *STAT4_rs7574865* and *CFB_rs12614* and PegIFNa treatment response.^{9,10} We found that the distributions of the genotypes of these two SNPs were very similar in PegIFNa-treated patients and NUCs-treated patients. Except for the above two SNPs, the genotype distributions of rs28371597 (including GG, GT, and TT) were roughly the same between patients received PegIFNa or NUCs therapies.

Complement regulator-associated SNPs were selected via the screening procedure

Through the screening process as shown in Supplementary Figure 1, nine SNPs were screened out from the complement regulatory genes, such as *CD55*, *CABPA*, *CFB*, *CFD*, *CR1* and *CINH* (Supplementary Table 3). The correlations between the alleles of the above nine SNPs and CHB treatments were assessed in the separate four cohorts. Among the nine SNPs, only rs28371597, located in the intronic region of *CD55*, showed significant correlation with CR in both the two PegIFNa cohorts (PegIFNa cohort 1: $p=0.01$, OR=3.72; PegIFNa cohort 2: $p=8.57 \times 10^{-3}$, OR=2.20; Table 2). However, in the separate four cohorts, no other SNPs were statistically correlated with CR in PegIFNa-treated patients. All nine SNPs did not show statistically significant correlations with CR in NUCs-treated patients.

Rs28371597 was correlated with the levels of CR and HBsAg loss in CHB patients received PegIFNa treatment

Rs28371597 possesses a G major allele and a T minor

allele. Because the T allele of rs28371597 is uncommon, the GT and TT genotypes were combined into the GT/TT genotype to proceed with the succeeding investigations. PegIFNa-treated patients with the rs28371597_GG genotype were found to have significantly lower CR rate compared to those with the rs28371597_GT/TT genotype (PegIFNa cohort 1: 25.73% vs. 53.85%, $p=0.03$; PegIFNa cohort 2: 18.73% vs. 32.65%, $p=0.02$; PegIFNa cohort 1 plus cohort 2: 20.29% vs. 37.10%, $p=2.00 \times 10^{-3}$; Fig. 1). When other basic factors, such as HBV genotype, HBV DNA level, rs7574865, and rs12614 were modulated, the correlation remained statistically significant ($p=0.04$, 0.04 and 0.02 for the PegIFNa cohort 1, 2, and their combination, respectively, Table 3 and Supplementary Table 4).

We also examined the levels of HBeAg seroconversion, HBV DNA and CR both in patients who carried the rs28371597_GG genotype and in patients who carried the rs28371597_GT/TT genotype for the given time periods. The seroconversion rate of HBeAg was always greater in rs28371597_GT/TT, and the average levels of HBV DNAs in patients with rs28371597_GT/TT were consistently lower since 24 weeks (Supplementary Fig. 2A–D). As for the CR rate, it was lower in patients with the rs28371597_GG genotype than that in patients with the rs28371597_GT/TT genotype since 48 weeks in the PegIFNa cohort 1. While in the PegIFNa cohort 2, it was always lower in the rs28371597_GG genotype carriers than in the rs28371597_GT/TT genotype carriers (Supplementary Fig. 2E–F). HBsAg loss was also compared between patients with different rs28371597 genotypes. The levels of HBsAg loss in patients that possessed the rs28371597_GG genotype were persistently lower than in patients that possessed the GT/TT genotype (PegIFNa cohort 1: 2.34% vs. 7.69%; PegIFNa cohort 2: 2.34% vs. 4.08%; PegIFNa cohort 1 plus cohort 2: 2.34% vs. 4.84%), although the difference did not reach statistical significance owing to the limited number of patients with HBsAg loss (Fig. 1D–F).

PGS was correlated with the levels of CR and HBsAg loss in CHB patients received PegIFNa treatment

Recently, our group used *CFB_rs12614* and *STAT4_rs7574865* to establish the PGS, and discovered that PGS became larger, the CR rate gradually increased.¹⁰ Here, we tried to add *CD55_rs28371597* into the previous PGS, aiming to improving the ability of predicting individual therapy efficiency. The favorable alleles of rs28371597, rs12614, and rs7574865 were T, C, and T, respectively. The number of the favorable alleles that the CHB patients carried reflected the level of PGS. As expected, the higher level of PGS, the higher CR rate was obtained (Fig. 2A–C). For example, we observed that the CR rates in all PegIFNa-treated patients who carrying 0, 1, 2, 3, 4, and 5 favorable alleles corresponded to 0.00%, 3.45%, 18.43%, 22.12%, 34.04% and 62.50%, respectively (p -trend= 7.94×10^{-6} ; Fig. 2C). PGS was significantly associated with CR even after the adjustment for other basic factors (Table 4 and Supplementary Table 5).

The association analysis of PGS with the level of HBsAg loss yielded similar results (Fig. 2D–F). The levels of HBsAg loss were rising with carrying increased favorable alleles in patients received PegIFNa treatment (favorable alleles with 0, 1, 2, 3, 4, and 5, corresponding to 0.00%, 0.00%, 1.36%, 3.33%, 4.26% and 12.50%, respectively; p -trend= 9.40×10^{-3} ; Fig. 2F). Even after the adjustment for other basic factors, the correlations of PGS with HBsAg loss still reached a statistically significant level in PegIFNa-treated patients (Supplementary Tables 6 and 7).

Table 1. Characteristics of the patients treated with PegIFNa and NUCs

Baseline variable	PegIFNa-treated patients			NUC-treated patients			p-value (PegIFNa cohort 1+2 vs. NUC cohort 1+2)
	PegIFNa cohort 1 (n=186)	PegIFNa cohort 2 (n=708)	PegIFNa cohort 1+2 (n=894)	NUC cohort 1 (n=553)	NUC cohort 2 (n=316)	NUC cohort 1+2 (n=869)	
Male sex (%)	151 (81.18)	512 (72.30)	663 (74.16)	450 (81.40)	239 (75.60)	689 (79.29)	0.01
Age, years; mean (SD)	28.44 (6.51)	29.74 (6.70)	29.47 (6.68)	30.13 (8.96)	31.94 (9.40)	30.79 (9.16)	5.93×10 ⁻⁴
Han ethnicity (%)	184 (98.92)	658 (92.90)	842 (94.18)	540 (97.70)	301 (95.30)	841 (96.78)	0.53
HBV genotype (%)							0.03
B	68 (36.56)	278 (40.40)	346 (38.70)	215 (38.90)	115 (36.40)	330 (37.97)	
C	116 (62.37)	403 (58.50)	519 (58.05)	335 (60.60)	199 (63.00)	534 (61.45)	
Others	2 (1.08)	8 (1.20)	10 (1.11)	3 (0.50)	2 (0.60)	5 (0.59)	
NA	0 (0.00)	19 (2.70)	19 (2.13)	0 (0.00)	0 (0.00)	0 (0.00)	
HBV DNA [#] , log ₁₀ IU/mL; mean (SD)	7.64 (1.29)	7.93 (0.76)	7.87 (0.90)	7.71 (1.07)	7.80 (0.90)	7.74 (1.01)	5.10×10 ⁻³
HBsAg [#] , log ₁₀ IU/mL; mean (SD)	3.96 (0.77)	4.28 (0.53)	4.21 (0.60)	4.20 (0.68)	4.18 (0.72)	4.19 (0.69)	0.49
HBeAg [#] , log ₁₀ PEIU/mL; mean (SD)	2.42 (0.98)	3.04 (0.56)	2.91 (0.72)	2.74 (0.64)	2.72 (0.62)	2.73 (0.63)	3.52×10 ⁻⁸
ALT [#] , x ULN; mean (SD)	4.58 (4.02)	4.54 (2.13)	4.55 (2.65)	4.30 (3.79)	3.40 (2.52)	3.98 (3.41)	1.05×10 ⁻⁴
STAT4_rs7574865 genotype (%)							0.89
GG	98 (52.69)	329 (46.47)	427 (47.76)	271 (49.01)	142 (44.94)	413 (47.50)	
GT	71 (38.17)	303 (42.80)	374 (41.83)	223 (40.33)	142 (44.94)	365 (42.00)	
TT	17 (9.14)	65 (9.18)	82 (9.17)	55 (9.95)	31 (9.81)	86 (9.90)	
NA	0 (0.00)	11 (1.55)	11 (1.23)	4 (0.72)	1 (0.32)	5 (0.60)	
CFB_rs12614 genotype (%)							0.85
CC	172 (92.47)	651 (91.95)	823 (92.06)	508 (91.86)	294 (93.04)	802 (92.29)	
CT	14 (7.53)	44 (6.21)	58 (6.49)	38 (6.87)	22 (6.96)	60 (6.90)	
TT	0 (0.00)	2 (0.28)	2 (0.22)	3 (0.54)	0 (0.00)	3 (0.35)	
NA	0 (0.00)	11 (1.55)	11 (1.23)	4 (0.72)	0 (0.00)	4 (0.46)	
CD55_rs28371597 genotype (%)							0.55
GG	171 (91.94)	598 (84.46)	769 (86.02)	491 (88.79)	278 (87.97)	769 (88.49)	
GT	12 (6.45)	48 (6.78)	60 (6.71)	42 (7.59)	22 (6.96)	64 (7.36)	
TT	1 (0.54)	1 (0.14)	2 (0.22)	1 (0.18)	1 (0.32)	2 (0.23)	
NA	2 (1.08)	61 (8.62)	63 (7.05)	19 (3.43)	15 (4.75)	34 (3.91)	

[#]Baseline level. PegIFNa, pegylated-interferon alpha; NUCs, nucleoside analogues; NA, not available; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; ULN, upper limit of normal.

Table 2. Associations of the nine complement regulators-associated SNPs with CR in the two PegIFNa cohorts and two NUCs cohorts

SNP	AI-lele [#]	PegIFNa cohort 1			PegIFNa cohort 2			NUCs cohort 1			NUCs cohort 2		
		MAF	OR (95% CI)	p	MAF	OR (95% CI)	p	MAF	OR (95% CI)	p	MAF	OR (95% CI)	p
rs12057769	A/G	0.16	1.17 (0.63-2.19)	0.61	0.20	1.06 (0.76-1.48)	0.74	0.19	1.14 (0.80-1.61)	0.47	0.20	1.20 (0.73-2.00)	0.47
rs4266889	A/G	0.27	1.12 (0.68-1.87)	0.65	0.31	0.92 (0.68-1.24)	0.57	0.27	0.95 (0.70-1.31)	0.77	0.32	1.10 (0.71-1.72)	0.67
rs28371597	T/G	0.04	3.72 (1.26-10.99)	0.01	0.04	2.20 (1.21-4.02)	8.57 × 10 ⁻³	0.04	0.88 (0.43-1.81)	0.73	0.04	0.66 (0.19-2.25)	0.50
rs6662070	G/A	0.40	1.11 (0.70-1.77)	0.65	0.43	1.00 (0.76-1.32)	1.00	0.41	1.04 (0.78-1.37)	0.80	0.44	0.94 (0.62-1.44)	0.78
rs1507765	A/C	0.41	0.98 (0.62-1.56)	0.93	0.39	1.22 (0.93-1.61)	0.16	0.41	0.89 (0.67-1.18)	0.41	0.39	0.99 (0.64-1.52)	0.96
rs1048709	A/G	0.31	1.18 (0.72-1.91)	0.52	0.33	0.89 (0.66-1.19)	0.42	0.34	0.90 (0.67-1.21)	0.48	0.29	1.04 (0.66-1.65)	0.87
rs541862	C/T	0.06	1.16 (0.46-2.91)	0.75	0.05	1.25 (0.69-2.26)	0.46	0.05	0.90 (0.48-1.71)	0.75	0.06	1.44 (0.66-3.13)	0.35
rs78364821	T/C	0.16	0.83 (0.43-1.60)	0.58	0.16	1.01 (0.69-1.48)	0.95	0.14	1.01 (0.67-1.50)	0.98	0.17	1.29 (0.75-2.19)	0.36
rs3826945	C/T	0.41	1.43 (0.84-2.44)	0.19	0.38	1.25 (0.90-1.73)	0.18	0.38	0.90 (0.64-1.27)	0.55	0.40	1.03 (0.63-1.70)	0.90

[#]Minor allele/Major allele. CR, combined response; SNP, single-nucleotide polymorphism; CHB, chronic hepatitis B; PegIFNa, pegylated interferon alpha; NUC, nucleos(t)ide analog; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.

Discussion

In this study, we examined the correlations of complement regulators-associated SNPs with responses to CHB treatments in four independent, retrospective, multicenter, well-characterized cohorts. We demonstrated that rs28371597 located in *CD55* intron was strongly correlated with the level of CR in patients received PegIFNa treatment but not NUCs treatment. The new PGS model composed of *CFB*_rs12614, *STAT4*_rs7574865, and *CD55*_rs28371597, was strongly linked with the levels of CR and HBsAg loss and predicted a better off-treatment outcome individually in PegIFNa-treated patients.

The complement system is a key element of the innate immune response and can be triggered through the classical, lectin, or the alternative pathway.¹⁸ All pathways converge to produce protein complexes (C3 convertases) which cleave and activate the core C3 component into C3a and C3b which is a component of the C5 convertase. C5 divides C5 into C5a and C5b, the latter forms a MAC with C6-C9 at last.¹⁹ When activated, the components of the complement system are crucial to fight pathogens, target invaders for destruction, and initiate inflammatory responses.²⁰ Regulation of the complement system is managed by C1INH, C4BP, complement factors B, D, H and I (CFB, CFD, CFH and CFI), CD35, CD46, CD55, and CD59.¹³ These molecules are either co-factors (CFH, C4BP, CD35 and CD46) in the CFI-mediated cleavage of C3b (and/or C4b) or can promote the disassembly (CD35 and CD55) of the existing C3 convertases.^{21,22} In addition, CFB and CFD both regulate the formation of C3 convertases.¹² These complement regulators work to guarantee that the rate and extent of complement activation are proportionate to the duration and strength of the activating signal.

Besides the importance as a regulator of the complement system, complement regulatory proteins modulate T cell function and survival. Some studies have reported that complement regulatory proteins have a role in the interaction between the complement system and T cell immunity, which had been widely considered as a crucial player in HBV clearance.²³⁻²⁵ For example, activation by CD46, a membrane-bound protein of the complement system, contributed to a strong proliferative response of activated T cells,²³ and induced IFN γ secretion for differentiation into Th1 cells.²⁴ The immunomodulatory role of CD59 in T cells has also been reported.²⁵ Our previous study indicated there was significant correlation of *CFB*_rs12614 with PegIFNa therapy response in HBeAg-positive CHB patients,¹⁰ which prompted us to explore the correlations of complement regulators-associated SNPs with CHB treatments in CHB patients. Only *CD55*_rs28371597, one of nine SNPs chosen from genes encoding for complement regulatory proteins, was shown to be strongly related to PegIFNa therapy response in patients with CHB.

CD55, also known as decay accelerating factor (DAF), is a cell surface molecule that is glycosylphosphatidylinositol (GPI)-anchored. CD55 is not only a complement inhibitor, but also an immune modulator. Liu *et al.* showed the mouse lacking the *Daf1* gene could significantly promote T cell responses with the increased IFN- γ and decreased IL-10 production.²⁶ Other investigators have found that down-regulation of CD55 in T cells enhances T cell proliferation, suggesting its close association with T cell responsiveness.²⁷ A study by Capasso *et al.* discovered that CD55 combined to its ligand CD97 and then induced T cell strong response involving increased T cell proliferation and up-regulation of activation markers CD69 and CD25.²⁸ Furthermore, CD55 was also found to inhibit natural killer (NK) cells.²⁹ The findings show that both innate and adaptive immune responses are regulated by CD55.

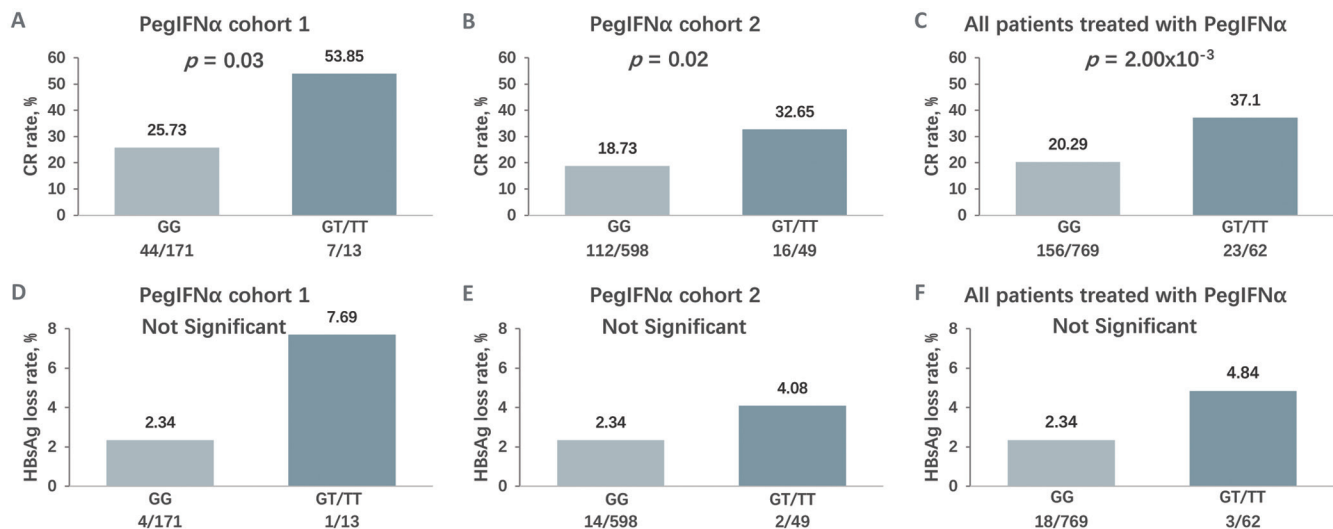


Fig. 1. CD55_rs28371597 is correlated with the levels of CR and HBsAg loss in the two PegIFNα cohorts. (A) CR in the PegIFNα cohort 1, (B) CR in the PegIFNα cohort 2, (C) CR in all the HBeAg-positive CHB patients treated with PegIFNα, (D) HBsAg loss in the PegIFNα cohort 1, (E) HBsAg loss in the PegIFNα cohort 2 and (F) HBsAg loss in all the HBeAg-positive CHB patients treated with PegIFNα. The level of CR or HBsAg loss in different CD55_rs28371597 genotypes is shown. Numbers under the genotypes denote the subjects with CR or HBsAg loss among all subjects with that genotype. *p*-values were calculated by the chi-squared test or Fisher's exact test. CR, combined response; HBsAg, hepatitis B s antigen; HBeAg, hepatitis B e antigen; PegIFNα, pegylated-interferon alpha.

Except for the direct antiviral effect, IFNα has another effect of immune modulation on both innate and adaptive immune response.³⁰ It is likely that CD55 links to IFNα treatment efficacy in an immunoregulatory manner. One possibility is that CD55 has influence on IFNα treatment via modulating T cell-mediated immunity, supported by the fact that IFNα regulates the T cell response which is important in establishing a functional cure of chronic HBV infection.³¹ Additionally, the effect of CD55 on IFNα treatment efficacy through regulation of NK cells seems rational and might be explained by the fact that HBV suppression during IFNα treatment coincides with a rise in the number of circulating CD56^{bright} NK cells, increased expression of the cytotoxic receptor TNF-related apoptosis-inducing ligand (TRAIL) by NK cells.^{32,33} Moreover, during the above actions of immune responses, CD55-mediated IFNγ production may also contribute to durable IFNα treatment re-

sponse.^{32,33} Taken together, CD55 may play an integral part in achieving the control of HBV infection with IFNα treatment response by regulating T cell immunity, NK cells activation and IFNγ production.

As mentioned above, rs28371597 is located in the intron region of CD55. Other than genetic polymorphisms in the coding region causing missense mutations which result in protein dysfunction, intronic polymorphisms may locate in the regulatory element sites, such as cis-regulatory element region, suggesting that they may have a biological role by regulating gene expression.³⁴ HaploReg v4.1 database shows that the regulatory motifs of several well-known transcription factors including DMRT4 and Foxp3, change because of the different alleles of rs28371597, thereby affecting their binding activities. In addition, rs28371597 may be just a tagging SNP, which is in high LD with the causal

Table 3. Multivariate logistic regression analysis of rs28371597 with CR in the two PegIFNα cohorts

	PegIFNα cohort 1		PegIFNα cohort 2		PegIFNα cohort 1+2	
	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>
Trial (PegIFNα cohort 1 vs. 2)						
Sex (Female vs. Male)						
Age, years			0.94 (0.91–0.98)	1.75×10 ⁻³	0.96 (0.93–0.99)	4.68×10 ⁻³
HBV genotype (B vs. C)			1.60 (1.06–2.40)	0.03	1.61 (1.12–2.33)	0.01
HBV DNA [#] , log ₁₀ IU/mL						
HBsAg [#] , log ₁₀ IU/mL					0.74 (0.52–1.04)	0.08
HBeAg [#] , log ₁₀ PEIU/mL	0.47 (0.32–0.69)	1.04×10 ⁻⁴	0.50 (0.35–0.72)	1.61×10 ⁻⁴	0.57 (0.43–0.76)	9.80×10 ⁻⁵
ALT [#] , log _e ×ULN	2.49 (1.42–4.36)	1.40×10 ⁻³			1.83 (1.29–2.58)	6.08×10 ⁻⁴
rs7574865 genotype	1.85 (1.09–3.12)	0.02			1.39 (1.07–1.80)	0.01
rs12614 genotype			4.19 (0.98–17.97)	0.05	3.85 (1.15–12.82)	0.03
rs28371597 genotype	3.52 (1.03–12.00)	0.04	2.01 (1.03–3.91)	0.04	1.99 (1.11–3.56)	0.02

[#]Baseline level. CR, combined response; PegIFNα, pegylated interferon alpha; OR, odds ratio; CI, confidence interval; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; ULN, upper limit of normal.

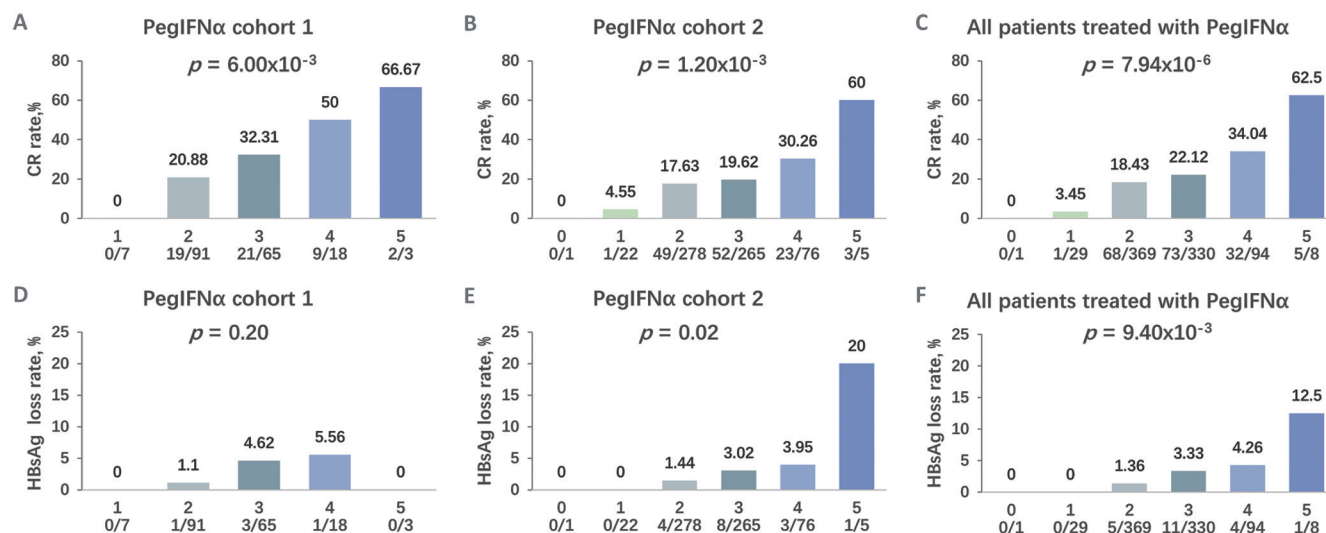


Fig. 2. PGS is correlated with the levels of CR and HBsAg loss in the two PegIFNa cohorts. (A) CR in the PegIFNa cohort 1, (B) CR in the PegIFNa cohort 2, (C) CR in all the HBeAg-positive CHB patients treated with PegIFNa, (D) HBsAg loss in the PegIFNa cohort 1, (E) HBsAg loss in the PegIFNa cohort 2 and (F) HBsAg loss in all the HBeAg-positive CHB patients treated with PegIFNa. The level of CR or HBsAg loss in different PGS values is shown. Numbers under the values denote the subjects with CR or HBsAg loss among all the subjects equal to that value. *p*-values were calculated by the Cochran-Armitage trend test. CR, combined response; HBsAg, hepatitis B s antigen; HBeAg, hepatitis B e antigen; PegIFNa, pegylated-interferon alpha; PGS, polygenic score.

SNP. One assumption is that rs28371597 or another causal SNP which is in high LD with rs28371597 affects *CD55* mRNA stability or protein synthesis via allele-imbalanced regulatory proteins, thus influencing its role for host immunity and then the response to IFNa treatment. Based on these studies, we hypothesized that the transcription factors DMRT4 or Foxp3 were less bound to the favorable allele T of rs28371597 and resulted in decreased expression of *CD55*. Subsequently, downregulation of *CD55* promoted T cell response and the number of circulating CD56^{bright} NK cells because of high IFN γ production and TRAIL expression. Finally, the T allele of rs28371597 had a role in the effectiveness of IFNa treatment (Fig. 3). Nevertheless, additional study is needed to discover how the *CD55* intronic SNP influences IFNa treatment efficiency.

The available evidence indicates that PGS has both dependable and clinically significant effectiveness.¹⁰ We previously constructed a PGS with only two SNPs to assess the combined impact of *CFB*_rs12614 with *STAT4*_rs7574865,

and found it effectively reflected clinical outcomes of CHB patients received PegIFNa treatment.¹⁰ We anticipate that when additional pharmaco-related SNPs are discovered, the PGS including more SNPs will be increasingly successful in predicting PegIFNa treatment response. In this study, we added *CD55*_rs28371597 into the previous PGS and constructed a new PGS model. The level of CR in PegIFNa-treated patients with the PGS model scored 5 was much higher than in patients with the PGS model scored less than 1 (62.5% vs. 3.45%). The level of HBsAg loss in PegIFNa-treated patients with the PGS model scored 5 was also much higher than in patients with the PGS model scored less than 1 (12.5% vs. 0.00%). Overall, a PGS can improve the ability of predicting individual therapy efficiency.

The differential performance of *CD55*_rs28371597 for predicting CHB therapy response to PegIFNa vs. NUCs is most probably owing to their distinct functional mechanisms. NUCs therapy suppresses HBV replication by targeting reverse transcriptase of HBV, but not through an immu-

Table 4. Multivariate logistic regression analysis of PGS with CR in the two PegIFNa cohorts

Parameters	PegIFNa cohort 1		PegIFNa cohort 2		PegIFNa cohort 1+2	
	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>
Trial (PegIFNa cohort 1 vs. 2)	–	–	–	–	–	–
Sex (Female vs. Male)	–	–	–	–	–	–
Age, years	–	–	0.95 (0.91–0.98)	2.32×10^{-3}	0.96 (0.93–0.99)	3.88×10^{-3}
HBV genotype (B vs. C)	–	–	1.63 (1.08–2.44)	0.02	1.66 (1.15–2.40)	6.57×10^{-3}
HBV DNA [#] , log ₁₀ IU/mL	–	–	–	–	–	–
HBsAg [#] , log ₁₀ IU/mL	–	–	–	–	0.74 (0.52–1.04)	0.08
HBeAg [#] , log ₁₀ PEIU/mL	0.48 (0.33–0.71)	2.05×10^{-4}	0.48 (0.33–0.69)	7.74×10^{-5}	0.56 (0.43–0.75)	6.49×10^{-5}
ALT [#] , log _e ×ULN	2.59 (1.48–4.55)	9.20×10^{-4}	–	–	1.87 (1.32–2.63)	3.65×10^{-4}
PGS	2.20 (1.39–3.50)	7.84×10^{-4}	1.47 (1.13–1.91)	4.21×10^{-3}	1.56 (1.24–1.95)	1.27×10^{-4}

[#]Baseline level. PGS, polygenic score; CR, combined response; PegIFNa, pegylated interferon alpha; OR, odds ratio; CI, confidence interval; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; ULN, upper limit of normal.

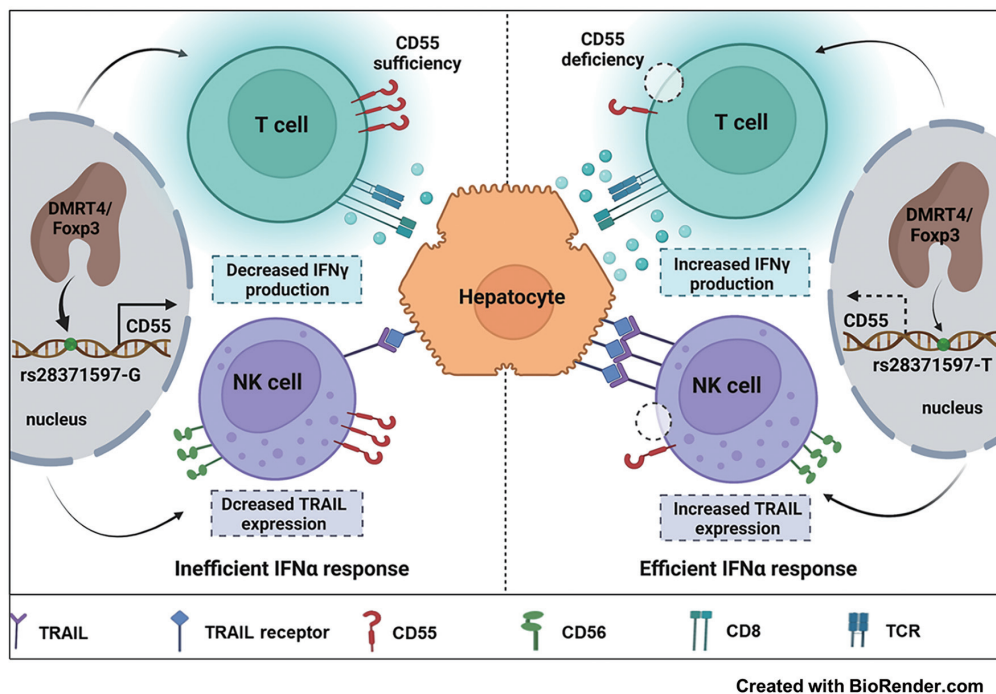


Fig. 3. Hypothesis for the molecular mechanism of the *CD55* polymorphism regulating PegIFNα therapy response.

noregulatory effect.⁴ The major effect of PegIFNα is immune response that targets HBV-infected hepatocytes, thus decreasing the number of cells containing the HBV cccDNA.³⁵ PegIFNα is a potential therapeutic agent with the advantage of achieving off-treatment outcomes probably because of the specific and efficient mechanism involving both immunomodulation and viral suppression.

It should be emphasized that this study has numerous limitations. First, the association of *CD55*_rs28371597 with HBsAg loss did not reach a statistically significant level in either the two PegIFNα cohorts or their combination. One explanation is that the relatively small sample of patients with HBsAg loss lacks sufficient statistical power to confirm the connection, and which may require additional validation by other larger investigations in the future. Second, because of the relatively small size of PegIFNα cohort 1, the PGS was statistically linked to HBsAg loss in PegIFNα cohort 2. Third, because the HBV genotypes in our patients were primarily genotype B and genotype C, the efficacy of rs28371597 with other HBV genotypes was not discovered and has to be explored further.

Conclusions

In Chinese HBeAg-positive CHB patients, *CD55*_rs28371597 was significantly correlated with the response to PegIFNα therapy. A PGS composed of *CFB*_rs12614, *STAT4*_rs7574865, and *CD55*_rs28371597 may satisfactorily predict clinical outcomes of CHB patients treated with PegIFNα, thereby assisting clinicians and patients in selecting the best treatment approach.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Contributed to study conception and design (DKJ, JC, HC), preparation of the draft of this manuscript (JC, SL, HC), analysis and interpretation of data (JC, HC, BZ), revision of the draft and supervision of the work (DKJ, JH, JS). All authors contributed to the article and approved the submitted version.

Ethical statement

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki (as revised in 2013), and the Nanfang Hospital Ethics Committee authorized this study. All patients signed an informed consent form.

Data sharing statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

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