RESEARCH ARTICLE

Single Nucleotide Polymorphism of *Interferon Lambda-4* Gene is not Associated with Treatment Response to Pegylated Interferon in Thai Patients with Chronic Hepatitis B

Umaporn Limothai[1], Rujipat Wasitthankasem[1],Yong Poovorawan[1], Pisit Tangkijvanich[2]*

Abstract

The single nucleotide polymorphism (SNP) ss469415590 in the *interferon lambda-4 (IFNL4)* gene has recently been reported to have an association with treatment response in chronic hepatitis C. However, any importance of the SNP in association with response to pegylated interferon (PEG-IFN) therapy in patients with chronic hepatitis B (CHB) is unclear. We retrospectively analyzed data for Thai patients with CHB treated with PEG-IFN for 48 weeks. Virological response (VR) for HBeAg-positive CHB was defined as HBeAg seroconversion plus HBV DNA level <2,000 IU/mL at 24 weeks post-treatment. VR for HBeAg-negative CHB was defined as an HBV DNA level <2,000 IU/mL at 48 weeks. The SNP was identified by real time PCR using the *Taq*Man genotyping assay with MGB probes. A total 254 patients (107 HBeAg-positive and 147 HBeAg-negative) were enrolled in the study. The distribution of TT/TT, ΔG/TT and ΔG/ΔG genotypes was 221 (87.0%), 32 (12.6%) and 1 (0.4%), respectively. Patients with non-TT/TT genotypes had significantly higher baseline HBV DNA levels than patients with the TT/TT genotype. In HBeAg-positive CHB, 41.2% of patients with TT/TT genotype versus 50.0% with non-TT/TT genotype achieved VR (P=0.593). In HBeAg-negative CHB, the corresponding figures were 40.3% and 43.5%, respectively (P=0.777). There was no significant correlation between the SNP genotypes and HBsAg clearance in both groups of patients. In summary, ss469415590 genotypes were not associated with response to PEG-IFN in Thai patients with HBeAg-positive and HBeAg-negative CHB.

Keywords: Chronic hepatitis B - single nucleotide polymorphism - *IFNL4* - pegylated interferon - treatment response

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Introduction

Hepatitis B virus (HBV) is a major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) worldwide (Gao et al., 2012). Pegylated interferon alfa (PEG-IFN), which has an immunomodulatory and antiviral effects, is one of the approved agents for treatment of patients with chronic hepatitis B (CHB) (Hoofnagle et al., 2007). The long-term therapeutic effect of PEG-IFN is durable and patients who achieve treatment response have a reduced risk of cirrhosis and HCC (Sung et al., 2008). However, the overall sustained response rate to PEG-IFN can be achieved in approximately 30-40% of patients with HBeAg-positive CHB and 20-30% of patients with HBeAg-negative CHB (Hoofnagle et al., 2007). In addition, PEG-IFN treatment is expensive and has potential side effects. Thus, selection of patients with a high probability of treatment response to PEG-IFN is important.

The outcome of therapy in patients with CHB is likely related to multiple factors, including viral factors and host genetic variations. Recent genome-wide association studies (GWAS) have reported that single nucleotide polymorphisms (SNPs) near the *IFNL3* gene (formerly known as *IL28B*) mainly rs12979860 was associated with both spontaneous hepatitis C virus (HCV) clearance (Thomas et al., 2009; Rauch et al., 2010) and response to PEG-IFN and ribavirin (RBV) treatment in patients with chronic hepatitis C (CHC) (Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Thomas et al., 2009; Rauch et al., 2010). However, the association of the SNPs with treatment response to PEG-IFN therapy in patients with CHB remains unclear (Stattermayer et al., 2014). Recently, Prokunina-Olsson et al. have discovered a dinucleotide variant ss469415590 (TT or ΔG) in the upstream region of *IFNL3*. The ΔG is one-base deletion cause of a frameshift, which in turn produces the full-length protein that is designated as IFNL4. In contrast to ΔG, TT variant does not produce IFNL4 protein. Genetic association data have showed that ΔG variant is associated with poorer clearance of HCV and response to PEG-IFN and RBV treatment (Prokunina-Olsson et al., 2013), and

*[1]Center of Excellence in Clinical Virology, Department of Pediatrics, [2]Research Unit of Hepatitis and Liver Cancer, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand *For correspondence: pisittkvn@yahoo.com*

ss469415590 is considered a better predictor of treatment response compared to rs12979860 (Bibert et al., 2013; Prokunina-Olsson et al., 2013). It is speculated that *IFNL4* polymorphisms might be a good therapeutic predictor for patients with CHB similar to that observed in patients with CHC. Currently, there has no report that examines whether ss469415590 is associated with response to PEG-IFN in patients with CHB and therefore, deserves further investigation.

Materials and Methods

Patients

Thai patients with HBeAg-positive or HBeAg-negative CHB, who were treated and completed a full course of PEG-IFN therapy between January 2010 and November 2014 at the King Chulalongkorn Memorial Hospital, Bangkok, Thailand were enrolled in this study. These patients were treated with either PEG-IFN-alfa2a (180 μg/week) or PEG-IFN-alfa2b (1.5 μg/kg body weight/week) for 48 weeks and followed up for a minimum of 24 weeks after therapy. All patients were seropositive for HBsAg for at least 6 months before therapy, had elevated serum alanine aminotransferase (ALT) levels and had elevated serum HBV DNA levels. Patients co-infected with hepatitis C virus and/or human immunodeficiency virus were excluded. Virological response (VR) for HBeAg-positive CHB was defined as HBeAg seroconversion plus HBV DNA level <2,000 IU/mL at 24 weeks post treatment. VR for HBeAg-negative CHB was defined as HBV DNA level <2,000 IU/mL at 48 weeks post treatment.

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. Written informed consents were obtained from all patients. The study was approved by the Ethics Committee, Faculty of Medicine, Chulalongkorn University.

Serological and virological assays

Qualitative HBsAg, HBeAg, anti-HBe and anti-HBs measurements were performed by commercially available enzyme-linked immunosorbent assays (Abbott Laboratories, Chicago, IL). HBsAg titers were quantified by Elecsys HBsAg II Quant reagent kits (Roche Diagnostics, Indianapolis, IN), according to the manufacturer's instructions. HBV DNA levels were quantified by Abbott RealTime HBV assay (Abbott Laboratories, Chicago, IL) with the lower limit of detection of 10 IU/mL. HBV genotypes were determined by direct sequencing, as previously described (Tangkijvanich et al., 2010).

Genotyping of ss469415590

Genomic DNA of CHB patients was extracted from 100 ml of the buffy coat using phenol-chloroform method. The SNP ss469415590 was identified by real time PCR using the TaqMan genotyping assay with MGB probes (Applied Biosystems) as described previously (Prokunina-Olsson et al., 2013; Akkarathamrongsin et al., 2014). Briefly, the real time mixture consisted of 1 μL of DNA extract, 200 nM of each probe (ss469415590_*IFNL4*_VIC: 5'-ATCGCAGAAGGCC-3' and ss469415590_IFNL4_FAM: 5'-ATCGCAGCGGCCC-3'), 900 nM of each primer (ss469415590_IFNL4_F: 5'-GCCTGCTGCAGAAGCAGAGAT-3' and ss469415590_IFNL4_R: 5'-GCTCCAGCGAGCGGTAGTG-3') (Apply Biosystem, NY), 5 μL of 2x Perfect Taq Plus MasterMix (5 PRIME, Gaithersburg, MD) adjusted to 10 μL final volume by distilled water. The PCR conditions were as follows: 2 minutes at 50°C, 10 minutes at 95 °C, 45 cycles of 15 seconds at 95 °C and 2 minutes at 60 °C. The fluorescent signal was detected at the end of each cycle. The genotype of ss469415590 was analyzed applying the End Point Genotyping method (LightCycler 480, Roche Diagnostics, IN). According to ss469415590 genotypes, TT was defined as major alleles and ΔG was defined as minor alleles, respectively.

Liver stiffness measurement

After fasting for at least 2 hours, liver stiffness measurement (LSM) was obtained before therapy using transient elastography (FibroScan, Echosens, Paris, France). Results were recorded in kilopascals (kPa) as the median value of all measurements. The procedure was based on at least 10 validated measurements: the success rate was over 60% and the interquartile range was less than 30% (Castera et al., 2012).

Statistical analysis

Statistical analysis was performed using SPSS version 22 software (SPSS, Chicago, IL, USA). The Mann-Whitney U test or Student's test were used to compare continuous variables, and the χ^2 test were used to compare categorical variables. Logistic regression was used to assess odd ratios of factors associated with VR. P-values<0.05 were considered statistically significant.

Results

Patient characteristics

Of the 281 consecutive patients with completed a full course of treatment and followed up, 254 patients had complete clinical data and blood samples available for genomic DNA isolation and were included in this study. There were 107 and 147 patients with HBeAg-positive and HBeAg-negative CHB, respectively. Table 1 summarizes demographic and clinical characteristics of the patients according to HBeAg status. Patients with HBeAg-positive CHB had a significantly lower mean age, but a higher proportion of male gender compared with HBeAg-negative CHB. Patients with HBeAg-positive CHB also had significantly higher baseline ALT, $\log_{10}$ HBV DNA and $\log_{10}$ HBsAg levels compared with HBeAg-negative CHB. There were no significant differences between groups in terms of HBV genotype distribution and baseline LSM. Among patients with HBeAg-positive CHB, VR was achieved in 45 (42.1%) patients and HBsAg clearance was achieved in 10 (9.3%) patients. Among patients with HBeAg-positive CHB, the corresponding figures were 60 (40.8%) and 11 (7.5%), respectively.

The prevalence of ss469415590 genotypes and treatment response

Table 1. Baseline Characteristics and Treatment Response of Patients According to HBeAg Status

Characteristics	HBeAg-positive CHB (n=107)	HBeAg-negative CHB (n=147)	P value
Age (year)	34.3 ± 8.3	41.3 ± 9.5	<0.001
Sex (male)	72 (67.3%)	78 (53.1%)	0.023
ALT (U/L)	116.9 ± 68.4	75.4 ± 35.9	<0.001
Log_{10} HBV DNA (IU/ml)	7.2 ± 1.0	5.4 ± 0.9	<0.001
Log_{10} HBsAg (IU/ml)	3.9 ± 0.6	3.4 ± 0.5	<0.001
HBV genotypes			0.639
B	11 (10.3%)	25(17.0%)	
C	63 (58.9%)	99 (67.3%)	
Other genotypes	1 (0.9%)	2 (1.4%)	
Missing	32 (29.9%)	21 (14.3%)	
Liver stiffness (kPa)	7.6 ± 4.2	6.8 ± 3.1	0.129
Virological response	45 (42.1%)	60 (40.8%)	0.843
HBsAg clearance	10 (9.3%)	11 (7.5%)	0.595

*ALT, alanine aminotransferase; Data described as means ± SD or n (%)

Table 2. Baseline Characteristics and Treatment Response of Patients According to ss469415590 genotypes

Characteristics	HBeAg-positive CHB		P value	HBeAg-negative CHB		P value
	TT/TT(n=97)	non-TT/TT (n=10)		TT/TT (n=124)	non-TT/TT (n=23)	
Age (year)	34.1 ± 7.8	36.6 ± 12.3	0.368	40.8 ± 9.6	43.6 ± 8.9	0.2
Sex (male)	68 (70.1%)	4 (40.0%)	0.053	65 (52.4%)	13 (56.5%)	0.717
ALT (U/L)	118.6 ±70.5	100.8 ± 41.6	0.436	73.9 ± 32.6	83.4 ±50.1	0.244
Log_{10} HBV DNA (IU/ml)	7.2 ± 0.9	7.6 ± 0.5	0.039*	5.4 ± 0.9	5.8 ± 0.9	0.042*
Log_{10} HBsAg (IU/ml)	3.9 ± 0.6	4.0 ± 0.4	0.543	3.4 ± 0.5	3.4 ± 0.4	0.496
Liver stiffness (kPa)	7.7 ± 4.3	6.4 ± 0.7	0.051	6.8 ± 3.1	6.4 ± 1.8	0.601
Virological response	40 (41.2%)	5 (50.0%)	0.593	50 (40.3%)	10 (43.5%)	0.777
HBsAg clearance	9 (9.3%)	1 (10.0%)	0.94	10 (8.1%)	1(4.3%)	0.534

*ALT, alanine aminotransferase; Data described as means ± SD or n (%)

Table 3. Logistic regression analysis of pretreatment factors to predict virological response

	Odd ratio	95% CI	P value
HBeAg-positive CHB			
Age (<40 years)	1.35	0.51-3.55	0.545
Gender (male)	0.67	0.29-1.52	0.342
ALT (>80 U/l)	1.37	0.63-2.97	0.427
Log_{10} HBV DNA (<7.0 IU/ml)	1.24	0.57-2.72	0.592
Log_{10} HBsAg (<4.0 IU/ml)	2.13	0.96-4.73	0.062
Liver stiffness (<7.5 kPa)	1.27	0.43-3.78	0.667
TT/TT genotype	0.7	0.19-2.59	0.594
HBeAg-negative CHB			
Age (<40 years)	0.84	0.43-1.62	0.598
Gender (male)	1.14	0.59-2.21	0.696
ALT (>80 U/l)	1.22	0.57-2.60	0.611
Log_{10} HBV DNA (<5.0 IU/ml)	1.16	0.59-2.27	0.657
Log_{10} HBsAg (<3.5 IU/ml)	2.62	1.31-5.24	0.007*
Liver stiffness (<7.5 kPa)	0.94	0.39-2.23	0.883
TT/TT genotype	0.8	0.35-1.85	0.603

The distribution of TT/TT, ΔG/TT and ΔG/ΔG genotypes in the entire cohort was 221(87.0%), 32(12.6%) and 1(0.4%), respectively. The distribution of the corresponding genotypes in patients with HBeAg-positive CHB were 97(90.7%), 10(9.3%) and 0(0%), respectively, and in patients with HBeAg-negative CHB were 124(4.3%), 22(15.0%) and 1(0.7%), respectively, which was not significantly different between groups (P=0.278).

Since a relatively small proportion of individuals displayed ΔG/TT or ΔG/ΔG genotypes, we grouped these genotypes together as non-TT/TT genotype for statistical

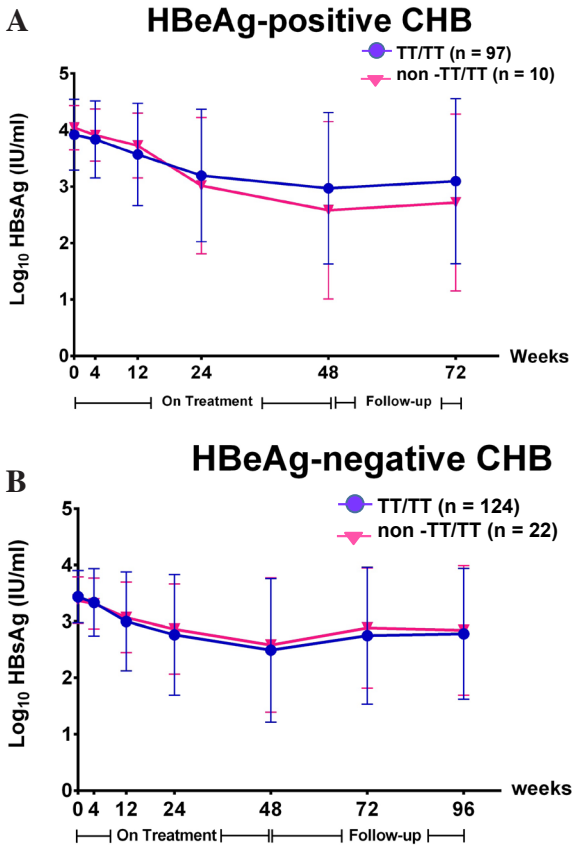

Figure 1. Serum HBsAg Kinetics in Relation to ss469415590 Genotypes in HBeAg-positive CHB (a) and HBeAg-negative CHB (b)

analysis. As shown in Table 2, there was no significant difference between patients with TT/TT and non-TT/TT genotypes in terms of age, sex, baseline ALT, $\log_{10}$ HBsAg level and LSM. However, patients with non-TT/TT genotypes had significantly higher HBV DNA levels than patients with TT/TT genotype in both HBeAg-positive and HBeAg-negative groups. In addition, patients with TT/TT and non-TT/TT genotypes had comparable rates of virological response and HBsAg clearance.

Serum HBsAg kinetics in relation to ss469415590 genotypes were also examined. In both HBeAg-positive and HBeAg-negative CHB, there was no difference in HBsAg decline from baseline during therapy through the end of follow-up between the TT/TT and non-TT/TT groups (Figure 1a and 1b).

Factors associated with virological response

To identify factors associated with VR, baseline characteristics of patients were analyzed by logistic regression analyses. Potential predictors of virological response included sex, age, ALT level, $\log_{10}$ HBV DNA level, $\log_{10}$ HBsAg level, liver stiffness and SNP ss469415590. In HBeAg-positive CHB, no factor was associated with VR. In HBeAg-negative CHB, low pretreatment HBsAg level was the only factor associated with VR. SNP ss469415590 genotype was not a predictor of VR in both HBeAg-positive and HBeAg-negative CHB (Table 3).

Discussion

It has been recognized that both host and virus factors can influence treatment response in patients with chronic viral hepatitis. Increasing data have suggested that host genetic variations may play an important role in the natural history and treatment outcome of patients with chronic viral hepatitis (Stattermayer et al., 2014). In patients with CHC, ss469415590 polymorphism has been shown to be the best predictor of response to PEG-IFN/RBV treatment and also the best marker of spontaneous HCV clearance (Bibert et al., 2013; Prokunina-Olsson et al., 2013). Such association might be in part explained by the biological activity of the IFNL4 protein that could induce the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway and the expression of interferon-stimulated genes (ISG) (Prokunina-Olsson et al., 2013). In addition, it has been shown that induction of *IFNL3* and IFN-γ inducible protein 10 (IP-10) mRNA relies on ss469415590, but not on rs12979860 of *IFNL3*, indicating that ss469415590 is the only functional variant identified so far associated with HCV clearance (Bibert et al., 2013). Moreover, the active IFNL4 protein is considered to be the driver of high hepatic ISG expression, which might be responsible for decreased clearance rates of HCV following PEG-IFN-based therapy (Terczynska-Dyla et al., 2014).

To our knowledge, this is the first report that examined the association between ss469415590 genotype and treatment response to PEG-IFN in patients with HBeAg-positive and HBeAg-negative CHB. Our results, however, did not find an association between the SNP genotype and treatment response in both groups of patients with CHB. Specifically, patients harboring TT/TT genotype had similar virological response rates to those with non-TT/TT genotype (ΔG/TT and ΔG/ΔG) (HBeAg-positive CHB: 41.2% vs. 50.0%, respectively; HBeAg-negative CHB: 40.3% vs. 43.5% respectively). Similar observations were found in respect to HBsAg clearance rates at the end of follow-up in HBeAg-positive and HBeAg-negative CHB (9.3% vs. 10% and 8.1% vs. 4.3%, respectively). In addition, the decline of serum HBsAg during and after PEG-IFN therapy was not significantly different between patients harboring TT/TT and non-TT/TT genotypes. These data suggest that the impact of the *IFNL4* polymorphism on the response to PEG-IFN-based therapy may be different among patients with CHB and CHC. Interestingly, among chronic HCV-infected patients, the polymorphism did not predict treatment response to therapy across all HCV genotypes. For instance, our recent data showed that ss469415590 was only associated with the treatment response to PEG-IFN/RBV in patients infected with HCV genotype 1, but not with HCV genotypes 3 and 6 (Akkarathamrongsin et al., 2014).

As there are no other data regarding the impact of *IFNL4* ss469415590 polymorphisms on treatment outcomes in patients with CHB, we decided to compare our results with previous reports in relation to rs12979860. Indeed, ss469415590 and rs12979860 polymorphisms are in strong linkage disequilibrium (LD in individuals of Asian or European ancestry, whereas this linkage disequilibrium is moderate in individuals of African populations (Prokunina-Olsson et al., 2013). In our previous study, the presence of the ΔG allele of ss469415590 was well-correlated with the unfavorable T allele of rs12979860, thereby indicating strong linkage disequilibrium between these two SNPs (Akkarathamrongsin et al., 2014). As a result, the absolute difference in association with treatment response between these two SNPs might probably be negligible and the identification of either polymorphism could be sufficient in Thai populations.

The association of rs12979860 polymorphisms with treatment response to PEG-IFN therapy in patients with CHB remains unclear with conflicting results among published studies. In HBeAg-positive CHB, a multicenter study including 11 Asian and European sites retrospectively analyzed the association of *IFNL3* rs12979860 genotypes with treatment response and demonstrated that CC (vs. CT/TT) genotype was independently associated with an increased probability of HBeAg clearance at 6 months post-treatment (Sonneveld et al., 2012). In another study conducted in Han Chinese populations, it was shown that *IFNL3* polymorphisms were independent predictors of treatment response to PEG-IFN in patients with HBeAg-positive CHB (Wu et al., 2015). In HBeAg-negative CHB, a study in Italian patients infected with HBV genotype D indicated that rs12979860 polymorphism was associated with HBsAg clearance following PEG-IFN treatment (Lampertico et al., 2013). In contrast, no association between the polymorphism and treatment response was observed in several studies in patients with HBeAg-positive and HBeAg-negative CHB (Tseng et al., 2011; de Niet et al., 2012; Holmes et al., 2013; Cheng et al., 2014;

Zhang et al., 2014). This inconsistency between reports is probably related to the heterogeneity of studies in terms of population, genetic background, HBV genotypes, sample size, treatment regimen, treatment endpoints and length of follow-up.

In conclusion, the current study suggested that the use of ss469415590 genotype might be limited as a predictive marker of treatment response to PEG-IFN in Thai patients chronically infected with CHB. Thus, the determination of *IFN4* polymorphism, as demonstrated in patients infected with HCV genotype 1, may not be useful in clinical practice. Nonetheless, the results of this study need to be confirmed with larger prospective cohorts to establish a potential association between ss469415590 genotype and response to PEG-IFN therapy in patients with HBeAg-positive and HBeAg-negative CHB.

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