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Association of *IFNAR2* and *IL10RB* genes in chronic hepatitis B virus infection

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Abstract

In this study, we investigated the effects of two functional polymorphisms, type I interferon receptor 2 gene (*IFNAR2*)-F8S and interleukin-10 receptor subunit beta gene (*IL10RB*)-K47E, on chronic hepatitis B virus (HBV) infection. We included 227 Thai patients with chronic HBV infection [100 with hepatocellular carcinoma (HCC) and 127 non-HCC], 170 individuals with self-limited HBV infection and 150 healthy controls. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to analyze these two single nucleotide polymorphisms (SNPs). In this study, the C allele of IFNAR2-F8S was found to be significantly increased in chronic HBV patients when compared with healthy controls [odds ratio, OR (95% confidence interval, CI) = 3.31 (2.11−5.21), $P = 6.214 \times 10^{-9}$ and corrected P-value, $P_c = 1.864 \times 10^{-8}$]. The effect of this allele was similar to that of an autosomal dominant gene in the presence of CC and CT genotype, when compared to TT with an OR of 4.02 ($P = 4.631 \times 10^{-9}$ and $P_c = 1.389 \times 10^{-8}$). Furthermore, AA genotype of IL10RB-K47E was found to be significantly decreased in chronic HBV patients compared with individuals with self-limited HBV infection ($P = 0.006$, $P_c = 0.018$ and OR = 0.45). For haplotype analysis, we found CA and CG haplotypes were associated with susceptibility to chronic HBV ($P = 0.014$, OR = 6.84 and $P = 0.002$, OR = 3.75, respectively) when compared with healthy individuals. This study suggests that IFNAR2-F8S polymorphisms might be involved in the susceptibility to chronic HBV infection. Moreover, AA genotype of IL10RB-K47E may provide a protective effect in this disease. However, an association study using a larger sample size should be performed to confirm these findings.

Introduction

Hepatitis B virus (HBV) infection is the most common cause of acute and chronic hepatitis. Although the number of HBV infection has been reduced by the use of an effective HBV vaccine, there remain 350 million individuals worldwide infected chronically and who become carriers of the virus. In addition, chronic HBV infection is associated with the development of cirrhosis and hepatocellular carcinoma (HCC) leading to the death of approximately 1 million people each year (1). Thailand has been classified as a region of intermediate endemicity as well as Taiwan, India, Pakistan, Korea and Philippines (2). Chinese twin studies showed a higher concordance rate for hepatitis B e antigen (HBeAg) persistence in monozygotic twins as compared with dizygotic twins (3). Therefore, the difference of host genetics might affect the different outcomes of patients with HBV infection. Several studies indicate an association between human leukocyte antigen gene (HLA) with this disease and/or the ability to eliminate HBV (4–6). However, recent studies showed that non-HLA genes are likely to be involved more than HLA genes. In a whole genome study of Frodsham et al., a cluster of class II cytokine receptor gene on chromosome 21q22 was identified as a major susceptibility locus (7). Further study of Frodsham et al. found IFNAR2-F8S and IL10RB-K47E that are nonsynonymous single nucleotide polymorphisms (SNPs) were significantly associated with outcome of persistent HBV infection in Gambians (7). Moreover, several studies in Chinese populations reported that these two functional SNPs were associated with susceptibility to chronic HBV infection (8, 9). However, these studies have shown controversial results. In

the Chen study (9), IL10RB SNP, A allele was found to be at risk for chronic HBV infection, whereas Gong et al's study (8) showed the protective effect of this allele. For IFNAR2 SNP, the study of Gong et al. found that CC (SS) genotype was associated with HBeAg negative patients and the T (F) allele was associated with the risk to high viral loads. However, these results were not found in Chen et al's study. Therefore, we investigated the effects of these two polymorphisms on chronic HBV infection in a Thai population.

Materials and methods

Subjects

Two hundred and twenty-seven Thai patients with chronic HBV infection from Chulalongkorn Memorial Hospital were recruited in this study. The diagnosis of chronic hepatitis B was established by seropositivity for hepatitis B surface antigen (HBsAg) over a 6-month period and did not have any other types of liver diseases such as chronic hepatitis C or alcoholic liver disease. In addition, all patients had elevated serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Patients with chronic HBV infection were further divided into two groups: without ($N = 127$; 50 women and 77 men: mean age $\pm$ SD $= 44.44 \pm 14.63$ years) and with HCC ($N = 100$; 15 women and 85 men: mean age $\pm$ SD $= 53.96 \pm 16.97$ years) according to the absence or presence of concurrent HCC. Diagnosis of HCC was based on histopathology and/or a combination of mass lesion in the liver from hepatic imaging and serum alpha fetoprotein level >400 ng/ml. Moreover, self-limited HBV group served as control for the population-based case–control study contained 170 subjects (89 women and 81 men: mean age $\pm$ SD $= 48.41 \pm 13.76$ years), who tested HBsAg negative and both HBV core antibody (anti-HBc) and HBV surface antibody (anti-HBs) positive, with normal liver function tests, and no history of HBV vaccination. Moreover, 150 ethnically and geographically matched controls (86 women and 64 men: mean age $\pm$ SD $= 24.27 \pm 7.19$ years) from healthy blood donor of the Thai Red Cross Society were recruited as healthy control group. The ethics committee of the faculty of Medicine, Chulalongkorn University, Bangkok, Thailand approved the study and the subjects gave their informed consent. Demographical characteristics and clinical profiles of subjects are shown in Table 1.

DNA extraction and genotyping study

DNA was extracted from the buffy coat collected with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, using the salting-out method (10). DNA was aliquoted and stored at -20°C until used. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to analyze the polymorphisms of IFNAR2 gene [F8S, rs2229207 (T/C)] and IL10RB gene [K47E, rs2834167 (A/G)]. The primer sequences for IFNAR2-F8S were newly designed in this study. The forward primer was 5′-CTATTCCTTACAGGTCTCTC-3′ and the reverse primer was 5′-GCAGCACTTACCCATGAG-3′. PCR conditions were as follows: predenaturation at 95°C for 2 min, followed by 35-step cycles of denaturation at 95°C for 30 s, annealing at 55°C for 50 s and extension at 72°C for 30 s, with a final extension at 72°C for 7 min; 10 µl of PCR products were digested with *Xmn*I for 16 h at 37°C. The digestion products were separated on 4% agarose gel and visualized under ultraviolet light with ethidium bromide staining. For IL10RB-K47E, analysis of this SNP was previously described by Chen et al. (9). Ten percent of the samples were confirmed by direct sequencing of PCR products to verify the accuracy of genotyping.

Statistical Analysis

Genotype frequencies were checked for consistency among normal controls with those expected from the Hardy–Weinberg equilibrium (HWE). Allele and genotype frequencies were compared between groups using the chi-squared (χ^2) test or Fisher's exact probability test, where appropriate. The PLINK v1.07 program was used to calculate HWE, P-values, odds ratios (ORs) and 95% confidence intervals (CIs), as well as for haplotype analysis (11). A P-value of <0.05 was considered statistically significant. In the case of multiple comparisons, corrected P-value (P_c) for a number of comparisons (Bonferroni correction) was applied.

Results

The distribution of genotype and allele frequencies of IFNAR2-F8S (rs2229207, T/C) and IL10RB-K47E (rs2834167, A/G) polymorphisms in chronic HBV patients (with HCC and without HCC), self-limited HBV patients and healthy controls is shown in Table 2. In this study, both SNPs were in HWE when comparing the observed and expected genotype frequencies of each SNP ($P > 0.05$).

In our study, we found statistically significant differences in the allele frequency of IFNAR2-F8S between patients with chronic HBV and healthy controls. The C allele of this SNP was significantly associated with an increased risk in chronic HBV infection as compared with healthy individuals [OR (95% CI) = 3.31 (2.11–5.21), $P = 6.214 \times 10^{-9}$ and $P_c = 1.864 \times 10^{-8}$]. The effect of C allele was similar to autosomal dominant in which the presence of CC and CT genotype when compared with TT conferred the OR of 4.02 (95% CI = 2.41–6.75, $P = 4.631 \times 10^{-9}$ and $P_c = 1.389 \times 10^{-8}$). In addition, we classified the total chronic HBV group into those with HCC ($N = 100$) and those without HCC ($N = 127$). Our finding showed a significant difference of C allele of

Table 1 Demographical characteristics and clinical profiles of subjects

	Healthy subjects	Self-limited HBV infection	Chronic HBV infection	
			Without HCC	With HCC
Number of subjects	150	170	127	100
Gender (female/male)	86/64	89/81	50/77	15/85
Age (mean ± SD)	24.27 ± 7.19	48.41 ± 13.76	44.44 ± 14.63	53.96 ± 16.97
ALT (U/l) (mean ± SD)	NA	NA	160.59 ± 225.95	61.86 ± 50.26
AST (U/l) (mean ± SD)	NA	NA	110.34 ± 136.84	87.6 ± 90.88
HBV DNA (log_{10}) (mean ± SD)	NA	NA	5.53 ± 1.77	5.75 ± 2.19

HBV, hepatitis B virus; HCC, hepatocellular carcinoma; ALT, alanine aminotransferase; AST, aspartate aminotransferase; NA, not applicable.

Table 2 Genotype and allele frequencies of *IFNAR2*-F8S and *IL10RB*-K47E gene polymorphisms in chronic HBV patients (with HCC and without HCC), self-limited HBV patients and healthy controls

SNP	Genotype/ allele	Total HBV $N = 227$ (%)	HBV with HCC $N = 100$ (%)	HBV without HCC $N = 127$ (%)	Self-limited HBV $N = 170$ (%)	Healthy control $N = 150$ (%)
IFNAR2-F8S	CC	13 (5.73)[a]	7 (7)[d]	6 (4.72)[f]	10 (5.88)	2 (1.33)
(rs2229207,T/C)	CT	96 (42.29)[a]	42 (42)[d]	54 (42.52)[f]	86 (50.59)	26 (17.33)
	TT	118 (51.98)	51 (51)	67 (52.76)	74 (43.53)	122 (81.33)
	C	122 (26.87)[b]	56 (28)[e]	66 (25.98)[g]	106 (31.18)	30 (10.00)
	T	332 (73.13)	144 (72)	188 (74.02)	234 (68.82)	270 (90.00)
IL10RB-K47E	AA	22 (9.69)[c]	13 (13)	9 (7.09)[h]	33 (19.41)	19 (12.67)
(rs2834167,A/G)	AG	115 (50.66)	51 (51)	64 (50.39)	67 (39.41)	69 (46.00)
	GG	90 (39.65)	36 (36)	54 (42.52)	70 (41.18)	62 (41.33)
	A	159 (35.02)	77 (39)	82 (32.28)	133 (39.12)	107 (35.67)
	G	295 (64.98)	123 (62)	172 (67.72)	207 (60.88)	193 (64.33)

HBV, hepatitis B virus; HCC, hepatocellular carcinoma; SNP, single nucleotide polymorphism.

[a]Autosomal dominant model CC + CT compared with TT genotype (total HBV *vs* healthy control); OR (95% CI) = 4.02 (2.41–6.75), $P = 4.631 \times 10^{-9}$, $P_c = 1.389 \times 10^{-8}$.

[b]C compared with T allele (total HBV *vs* healthy control); OR (95% CI) = 3.31 (2.11–5.21), $P = 6.214 \times 10^{-9}$, $P_c = 1.864 \times 10^{-8}$.

[c]AA compared with AG + GG genotype (total HBV *vs* self-limited HBV); OR (95% CI) = 0.45 (0.24–0.83), $P = 0.006$, $P_c = 0.018$.

[d]Autosomal dominant model CC + CT compared with TT genotype (HBV with HCC *vs* healthy control); OR (95%CI) = 4.19 (2.29–7.70), $P = 6.734 \times 10^{-7}$, $P_c = 2.020 \times 10^{-6}$.

[e]C compared with T allele (HBV with HCC *vs* healthy control); OR (95% CI) = 3.50 (2.09–5.87), $P = 2.630 \times 10^{-7}$, $P_c = 7.890 \times 10^{-7}$.

[f]Autosomal dominant model CC + CT compared with TT genotype (HBV with HCC *vs* healthy control); OR (95% CI) = 3.90 (2.20–6.94), $P = 3.814 \times 10^{-7}$, $P_c = 1.144 \times 10^{-6}$.

[g]C compared with T allele (HBV without HCC *vs* healthy control); OR (95% CI) = 3.61 (1.93–5.20), $P = 9.169 \times 10^{-7}$, $P_c = 2.751 \times 10^{-6}$.

[h]AA compared with AG + GG genotype (HBV without HCC *vs* self-limited HBV); OR (95% CI) = 0.32 (0.13–0.723), $P = 0.003$, $P_c = 0.009$.

IFNAR2-F8S (rs2229207) between chronic HBV patients with HCC *vs* healthy control [OR (95% CI) = 3.50 (2.09–5.87), $P = 2.630 \times 10^{-7}$ and $P_c = 7.890 \times 10^{-7}$] and the patients without HCC *vs* healthy controls [OR (95% CI) = 3.61 (1.93–5.20), $P = 9.169 \times 10^{-7}$ and $P_c = 2.751 \times 10^{-6}$]. The effect of C allele seems to be autosomal dominate with an OR of 4.19 (95% CI = 2.29–7.70, $P = 6.734 \times 10^{-7}$ and $P_c = 2.020 \times 10^{-6}$) for patients with HCC and the OR of 3.90 (95% CI = 2.20–6.94, $P = 3.814 \times 10^{-7}$, $P_c = 1.144 \times 10^{-6}$) in the patients without HCC.

For IL10RB-K47E, AA genotype was found to be significantly decreased in chronic HBV patients compared with those with self-limited HBV infection [OR (95% CI) = 0.45 (0.24–0.83), $P = 0.006$ and $P_c = 0.018$]. When we classified total chronic HBV patients into those with HCC and those without HCC, we found that only the chronic HBV patients without HCC still showed a significant decrease in the frequency of AA genotype as compared with the self-limited HBV group [OR (95% CI) = 0.32 (0.13–0.72), $P = 0.003$, $P_c = 0.009$].

Furthermore, we performed haplotype analysis of these two SNPs of IFNAR2-F8S and IL10RB-K47E. The results of haplotype analysis are shown in Table 3. There were four haplotypes including CA, TA, CG and TG. To test the association of IFNAR2-IL10RB haplotype and disease development, we compared each tested haplotype with the other three haplotypes between patient groups and healthy controls. In this study, we found significant association between CA and CG haplotypes and risk to chronic HBV infection [total chronic HBV (OR = 6.84 and $P_c = 0.014$), with HCC (OR = 7.7 and $P_c = 0.016$), without HCC (OR = 5.99 and $P_c = 0.039$); total chronic HBV (OR = 3.75 and $P_c = 0.002$), with HCC

Table 3 Haplotype analysis for *IFNAR2-F8S* (rs2229207) and *IL10RB-K47E* (rs2834167)

	Haplotype frequency				HBV without HCC *vs* Healthy control		HBV with HCC *vs* Healthy control		Total HBV *vs* Healthy control	
Haplotype	Healthy control	HBV without HCC	HBV with HCC	Total HBV	OR	P_c value	OR	P_c value	OR	P_c value
CA	0.0248	0.0730	0.0841	0.0835	5.99	**0.039**	7.7	**0.016**	6.84	**0.014**
TA	0.3416	0.2498	0.2896	0.2902	0.63	0.116	0.80	0.887	0.69	0.732
CG	0.0779	0.1868	0.1852	0.1858	3.72	**0.001**	3.48	**0.002**	3.75	**0.002**
TG	0.5557	0.4904	0.4412	0.4406	0.72	0.414	0.59	**0.048**	0.66	0.059

HBV, hepatitis B Virus; HCC, hepatocellular carcinoma; OR, odds ratio; P_c, corrected *P*-value.

(OR = 3.48 and $P_c = 0.002$), without HCC (OR = 3.72 and $P_c = 0.001$), respectively]. In contrast, TG was observed to be a protective haplotype. The protective association of TG haplotype was found only in chronic HBV patients with HCC when compared with healthy controls (OR = 0.59 and $P_c = 0.048$).

Discussion

In this study, we investigated the association between of IFNAR2-F8S (rs2229207, T/C) and IL10RB-K47E (rs2834167, A/G) polymorphisms and the susceptibility to chronic HBV infection. Our results suggest that the IFNAR2-F8S polymorphism might be important in the susceptibility to chronic HBV infection. In this study, we found that the C allele (S allele) of IFNAR2-F8S was higher in the chronic hepatitis B group when compared with healthy controls, but not when compared with the self-limited HBV group. The association of this SNP seems to be a dominant effect by model of inheritance analysis. Our results were consistent with previous Chinese studies of Chen et al. (9) and Gong et al. (8) who found no differences in the allele frequency in chronic HBV infection patients when compared to patients with self-limited HBV. However, these studies did not compare chronic HBV patients with healthy individuals. Our study is the first to report comparisons with healthy controls, which indicates susceptibility to chronic HBV. It is interesting that the genotype frequency between healthy controls and self-limited HBV differ. Our finding suggests that this SNP might be influential in individuals susceptible to HBV infection and lead to chronic HBV infection. The negative association in patients with self-limited HBV suggests that this SNP might not be important for viral clearance. In addition, we divided chronic HBV patients into patients with HCC and patients without HCC groups. Our results showed that IFNAR2-S allele was associated with both groups when compared with healthy controls. This suggests that the IFNAR2 polymorphism affect susceptibility to the chronic HBV infection but not the progression of HCC. *IFNAR2* gene is a member of class II cytokine receptor family located on chromosome 21 (12). The IFNAR2-F8S (rs2229207, T/C) is nonsynonymous SNP altering amino acid from phenylalanine to serine. This SNP is located on signal peptide region, which is important in IFNAR2 protein trafficking to the membrane. The risk S allele found in this study associates with lower cell surface protein expression of IFNAR2 (7). Furthermore, the enhancement of signal transduction and antiviral response induced by interferon-α (IFN-alpha) is lesser in cells expressing the S than the F allele (7). These observations suggest that S allele of IFNAR2 gene might be responsible for the risk of chronic HBV infection and the development of HCC by affecting the protein expression of IFNAR2 on the cell surface, leading to impaired signal transduction and antiviral response. However, the functionality of this polymorphism in our Thai patients should be further determined in more like studies.

For IL10RB-K47E (rs2834167, A/G), we did not find the association of allele frequency of this SNP between chronic HBV patients and healthy controls. However, when we analyzed genotype frequencies, the AA (KK) genotype was significantly decreased in chronic HBV patients when compared with patients with self-limited HBV infection. In addition, we observed that only the chronic HBV without HCC group showed a significant decrease in the frequency of KK genotype compared with patients with self-limited HBV infection when the total chronic HBV was classified into with and without HCC subgroups. Our results are consistent with a previous study suggesting that the KK might be a protective genotype in patients with persistent infection (8). However, these results differed from the study of Chen et al. (9) who found that the A allele may be a risk for chronic HBV infection. However, further study in other populations might confirm these finding. IL10RB-K47E is a nonsynonymous SNP changing amino acid from lysine to glutamic acid. This SNP is located on the first extracellular FnIII domain repeat region. There has been report that K allele of IL10RB gene affected lower receptor expression, resulting in the decrease of signal transduction (7). The reduced IL-10/IL-10R signal might cause the blockade of immunoregulatory role of IL-10 and lead to the resolution of chronic HBV infection. Further study is required to prove this hypothesis.

In haplotype analysis, we found that all haplotypes having risk C allele from IFNAR2 gene (CA and CG haplotypes) showed significant association with susceptibility to chronic HBV infection in both the patients with HCC and without HCC. This confirmed the importance of C allele in disease

susceptibility and the progression of HCC. However, these results are inconsistent with the study of Gong et al. (8) in which no significant associations were found by haplotype analysis. Increased sample sizes and further studies are needed to confirm this finding.

In conclusion, we found an association between IFNAR2-F8S polymorphisms and susceptibility to chronic HBV infection. Furthermore, a protective effect of AA genotype of IL10RB-K47E was observed in this study. Nevertheless, an association study using a larger sample size should be performed to further verify our findings.

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Conflict of Interests

The authors have declared no conflicting interests.

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