Association of cytokine and cytokine receptor gene polymorphisms with the risk of chronic hepatitis B

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Summary

Background: **Hepatitis B (HBV) infection is a major cause of chronic liver diseases, and the polymorphisms of cytokine genes may affect the progression of HBV-related hepatitis.**

Objective: **The aim of this study was to examine the association of cytokine polymorphisms with the susceptibility to HBV-related chronicity.**

Methods: **Specifically, a LIFECODES Cytokine SNP Typing kit was used to investigate 22 cytokine single nucleotide polymorphisms (SNPs) from 14 cytokine and cytokine receptor genes with the aim of analyzing the role of Th1 and Th2 genotype combination. This population-based case-control association study included 131 chronic HBV patients and a control group of 142 healthy donors.**

Results: **When the combination of Th1 and Th2 genotypes was analyzed for the genetic risk factor for chronic hepatitis B, we did not observe any significant association. A non-significant association betweenTh1 and Th2 and this risk factor could have resulted from the limitation of our small sample size. When the results from each genotype were separately analyzed, the frequencies of the heterozygous CA (-592) and CT (-819) genotype of IL-10 gene-promoter polymorphisms were significantly higher in chronic HBV patients than that in healthy controls (OR=1.76, 95%CI =1.03-3.01, *p* =0.028; OR=1.79, 95%CI =1.04-3.06, *p* =0.024, respectively). Interestingly, the TCC (-1098/-590/-33) haplotype frequency of *IL-4* showed a positive association with chronic hepatitis B as a protective haplotype (OR =0.53, 95%CI =0.32-0.85, *p* =0.005).**

Conclusion: **These preliminary results suggest that polymorphisms in some cytokine genes, particularly the Th2 cytokine, influence persistence of HBV infection.** ***(Asian Pac J Allergy Immunol 2013;31:277-85)***

Key words: *Hepatitis B virus, chronic HBV infection, single nucleotide polymorphism, cytokine, cytokine receptor*

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Introduction

Hepatitis B (HBV) infection is one of the most frequent viral infections and major global public health problems. In 2011, according to the World Health Organization, over 2 billion persons had been infected with the hepatitis B virus, one sixth of whom are chronically infected. Among these, Asians including those who migrate to other countries are found to have higher rates of infection. After primary HBV infections, the immune system fails to eliminate the virus and allows virally persistent infection in approximately 5% of adults and 95% of neonates.[1] HBV is also the most common cause of chronic liver disease affecting more than 400 million people or approximately 6% of the world's population, especially in Asia and Africa.[2,3] In addition, approximately1 million patients suffer from the fatal development of cirrhosis and hepatocellular carcinoma.

HBV clearance is mediated by antiviral cytokines produced by immune cells of the innate and adaptive immune response, especially interferon

(IFN)-α/β/γ) and tumor necrosis factor alpha (TNF-α).[4] HBV-specific T-helper 1 cells that produce Th1 cytokines are involved in HBV clearance in both acute and persistent infection.[5] Both viral (viral load, genotype and genomic mutations) and host factors (age, sex and immune status) contribute to differential clinical outcomes. As a result, both the cytokine polymorphisms that dictate the functionality of cytokine and the immune response might be associated with different outcomes of HBV infection.[6] By investigating these factors, healthcare practitioners could predict the severity of the cases and thus suggest the most effective treatment options to the patients.

Several HBV association studies of the human leukocyte antigen (HLA) genes, the single nucleotide polymorphisms (SNP) and the promoter region cytokine genes have been reported.[7-9] However, the cytokines work as a network *in vivo* and the association of several cytokines rather than with single cytokine lead to different outcomes. Here, we report the effects of 22 SNPs from 14 cytokine and cytokine receptor genes on the susceptibility to HBV-related chronicity among Thai patients with chronic HBV infection and healthy individuals. Most of these SNPs were putative functional SNPs and have been previously reported to be associated with chronic HBV infection by individual SNPs or genes but have not been analyzed to investigate the relationship between the SNPs of Th1 and Th2 cytokine genes as a network.

Methods

Subjects

This study protocol had been reviewed and approved by the ethics committee of the faculty of Medicine, Chulalongkorn University. This case–control study was comprised of 131 Thai chronic hepatitis B patients (male:female = 75:56, mean age = 50.2±12.7, age range = 25-81) at the Chulalongkorn Memorial Hospital and 142 ethnically and geographically matched healthy controls (male:female = 82 : 60, mean age = 30.8 ± 10.7, age range = 20-55) among the blood donors at the Thai Red Cross Society. After potential subjects completed an informed consent form, the presence of HBsAg, the level of serum alanine transaminase (ALT) and aspartate transaminase (AST) and the histopathology of their samples were examined. Apart from a positive test for HBsAg in a commercially available enzyme-linked immunosorbent assay kit (Abbott Laboratories, Chicago, IL) for at least six months, individuals whose samples showed an abnormal level of ALT and AST and the characteristic histopathology were categorized as chronically infected with hepatitis B. None of the chronic hepatitis B patients have hepatocellular carcinoma. All the samples were negative for anti-HIV and anti-HCV antibodies.

DNA extraction

Genomic DNA was isolated from peripheral blood leukocytes via a standard salting-out method that use dethylene diamine tetra acetic acid (EDTA) as an anticoagulant.[10]

Genotyping study

The genotyping study of 22 cytokine SNPs from 14 cytokine and cytokine receptor genes was performed by LIFECODES Cytokine SNP Typing kit to analyze *IL1A* -889T/C (rs1800587), *IL1B* -511C/T (rs16944), *IL1B* +3962T/C (rs1143634), *IL1R*(pst1 1970 C/T (rs2234650), *IL1RA* mspa1 11100 T/C (rs315952), *IL4RA*+1902 G/A (rs1801275), *IL12* -1188 A/C (rs3212227), *IFNG*+874 A/T (rs2430561), *TGFB* codon 10 T/C (rs1800470), *TGFB* codon 25 G/C (rs1800471), *TNFA*-308 G/A (rs1800629), *TNFA* -238 G/A (rs361525), *IL2*-330 T/G (rs2069762), *IL2*+166 G/T (rs2069763), *IL4*-1098 T/G (rs2243248), *IL4* -590 C/T (rs2243250), *IL4*-33 C/T (rs2070874)), *IL6* -174 G/C(rs1800795), *IL10*-1082A/G (rs1800896), *IL10*-819C/T (rs1800871), *IL10* -592C/A (rs1800872) and *IL18* -137C/G (rs187238). LIFECODES Cytokine-SSO typing kits utilize sequence-specific oligonucleotides (SSOs) to genotype the cytokine loci present in the PCR amplified samples. The loci included in the kit are putative functional SNPs. It is based on the hybridization of labeled single stranded PCR products to SSO probes. Firstly, the all cytokine loci were amplified by dividing them in four amplification reactions that use the cytokine master mix1-4. Then the amplified DNA product was hybridized using a probe. The probe mix contains two SSOs for each SNP that preferentially hybridizes to one of the alleles of a locus that may or may not be present in the amplified DNA. A different SSO probe can be attached to each color microsphere having up to 100 different populations of Luminex microspheres by its unique fluorescence signature or color so that they can be mixed together and analyzed by the Luminex instrument. After the hybridization step, the R-Phycoerythrin conjugated Streptavidin (SA-PE) was immediately added in the reaction and moved to the Luminex instrument for analysis.

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) was examined using a 2×2 χ^2 test comparing observed and expected numbers. To perform allele and genotype frequencies in the case-control association tests, the PLINK v1.07 program[11] was used to calculate the odds ratios, 95% confidence interval and *p* value. A *P* value of less than 0.05 was considered to be statistically significant. Haplotype frequencies were estimated using PHASE calculation software.[12]

Results

The genotypic distributions of each of the cytokine SNPs in chronic HBV patients and the healthy control, group except for *IL6* (-174) in the control group, was in Hardy-Weinberg equilibrium. That *IL6 (-174)* was not in the equilibrium was possibly because of the small sample size. There were no statistically significant differences in allelic distributions for any of the SNPs between chronic hepatitis B patients and healthy controls (Table 1). When the results from each genotype were separately analyzed, the frequencies of the heterozygous CA (-592) and CT (-819) genotypes of *IL10* gene promoter polymorphisms were significantly higher in chronic HBV patients than that in healthy controls (OR =1.76, 95%CI =1.03-3.01, *p* =0.028; OR=1.79, 95%CI =1.04-3.06, *p* =0.024, respectively) (Table 2). In addition, there was a trend of a positive association between chronic HBV and some cytokine gene polymorphisms such as *TNFA* -308A (OR =0.61 95%CI =0.34-1.09, *p* =0.09) and -590CT compared to TT (OR=0.61 95%CI=0.35-1.06, *p* =0.063). Interestingly, the TCC (-1098/-590/-33) haplotype frequency of *IL-4* showed an association with chronic hepatitis B as a protective haplotype (OR =0.53, 95%CI =0.32-0.85, *p* =0.005), as shown in Table 2.

In addition, we analyzed particular functional SNPs based on the types of cytokines to Th1 and Th2 cytokine and analyzed their distributions in the chronic HBV and control groups. Table 3 shows the association of genes based on the number of low activity genotypes of Th1 cytokine (*IFNG*, *IL12* and *IL18*) and Th2 cytokine (*IL4* and *IL10*), and table 4 shows the combined effect of Th1 and Th2 genotypes on the risk of chronic HBV. The analysis revealed that there was no statistically significant associations.

Discussion

The polymorphisms of cytokine and cytokine receptor genes influence their functionality through gene expression, mRNA stability or protein structure and have evident pathological consequences, including higher susceptibility to infection and number of chronic diseases.[13] In this study, we investigated the association between 14 cytokine and cytokine receptor genes and their relevance to individuals' susceptibility to chronic HBV infection. Comparisons with previous studies in other population are summarized below.

Pro-inflammatory cytokines

- ***TNFA***

Early association studies of *TNFA* showed mixed results. One study suggested that the -308 G/A polymorphism might play a role in the altered *TNFA* gene expression observed *in vitro,*[14] while another study proposed that the position -308 had no functional relevance for *TNFA* promoter transcription.[15] Since then, numerous association studies of its polymorphisms, including those related to hepatitis B, have been reported and ethnicity has been reported to influence the severity of HBV infection. At the position of -308 where allele G is more frequently found than allele A, the GG genotype (low activity) was less commonly found in chronic HBV patients with an OR=0.37 in the Brazilian population.[16] On the other hand, the G allele and GG genotype were believed to be indicative of high risk of HBV persistence with an OR=1.35 among East Asians in the meta-analysis.[17] As to *TNFA* -238G/A, a meta-analysis showed that G is less frequently found in chronic HBV (OR=0.92) but not to a statistically significant extent.[17] Our study revealed the same trend in the Thai population as the meta-analysis among the East Asians previously showed.

- ***IL1B***

We have previously shown that the CC genotype was higher in HBV associated HCC patients with a OR =1.20 (CI =0.80-1.79)[18] and an OR =1.72 (CI =1.04-2.84, P =0.033),[19] whereas, in this study, there was no association with the *IL1B* -511 position. However, a previous published study in the Chinese population and our study showed that genotype distributions and allelic frequencies for *IL1B* (-511) promoter polymorphisms in patients with chronic hepatitis B and control subjects were not statistically different.[20]

Table1. Allelic distributions of cytokine and cytokine receptor polymorphisms in patients in chronic HBV patients and healthy controls

Genes	SNPs	allele	CHB n (%)	Control n (%)	OR (95% Confidence Interval)	*P*
TNFA	-308	G	240(92.3)	248(87.9)	1.00	**0.090**
	(rs1800629)	A	20(7.7)	34(12.1)	0.61(0.34-1.09)	
	-238	G	249(95.8)	276(97.2)	1.00	0.370
	(rs361525)	A	11(4.2)	8(2.8)	1.52(0.60-3.85)	
IL1A	-889	C	239(91.9)	264(93.0)	1.00	0.648
	(rs1800587)	T	21(8.1)	20(7.0)	1.16(0.61-2.19)	
IL1B	-511	C	135(51.9)	147(51.8)	1.00	0.970
	(rs16944)	T	125(48.1)	137(48.2)	0.99(0.71-1.39)	
	+3962	C	253(97.3)	280(98.6)	1.00	0.288
	(rs1143634)	T	7(2.7)	4(1.4)	1.94(0.56-6.69)	
IL1R	pst1 1970	C	179(68.3)	176(62.0)	1.00	0.120
	(rs2234650)	T	83(31.7)	108(38.0)	0.76(0.53-1.08)	
IL1RA	mspa1 11100	T	133(0.5)	151(53.1)	1.00	0.46
	(rs315952)	C	131(0.5)	133(46.9)	1.14(0.81-1.59)	
IL6	-174	G	257(98.1)	279(98.2)	1.00	0.898
	(rs1800795)	C	5(1.9)	5(1.8)	1.09(0.31-3.79)	
TGFB	Codon 10	C	98(50.0)	137(56.1)	1.00	0.199
	(rs1800470)	T	98(50.0)	107(43.9)	1.28(0.88-1.87)	
	Codon 25	G	240(99.2)	239(99.6)	1.00	0.567
	(rs1800471)	C	2(0.8)	1(0.4)	1.99(0.18-22.11)	
IL2	-330	T	155(62.5)	179(63.9)	1.00	0.734
	(rs2069762)	G	93(37.5)	101(36.1)	1.06(0.75-1.52)	
	+166	G	157(60.8)	175(61.6)	1.00	0.855
	(rs2069763)	T	101(39.2)	109(38.4)	1.03(0.73-1.46)	
IFNG	+874	A	191(73.5)	204(72.3)	1.00	0.769
	(rs2430561)	T	69(26.5)	78(27.7)	0.94(0.65-1.38)	
IL12	-1188	A	133(51.1)	154(54.6)	1.00	0.421
	(rs3212227)	C	127(48.9)	128(45.4)	1.15(0.82-1.61)	
IL18	-	G	224(86.2)	251(88.4)	1.00	0.515
	137(rs1872380)	C	36(13.8)	33(11.6)	1.22(0.72-2.09)	
IL4	-1098	T	236(90.8)	265(93.3)	1.00	0.273
	(rs2243248)	G	24(9.2)	19(6.7)	1.42(0.76-2.66)	
	-590	T	204(78.5)	206(72.5)	1.00	0.109
	(rs2243250)	C	56(21.5)	78(27.5)	0.73(0.49-1.08)	
	-33	T	202(77.1)	206(72.5)	1.00	0.220
	(rs2070874)	C	60(22.9)	78(27.5)	0.78(0.53-1.16)	
IL4RA	+1902	A	195(75.0)	208(74.8)	1.00	0.962
	(rs1801275)	G	65(25.0)	70(25.2)	0.99(0.67-1.46)	
IL10	-1082	A	245(94.2)	267(94.0)	1.00	0.915
	(rs1800896)	G	15(5.8)	17(6.0)	0.96(0.47-1.97)	
	-819	T	168(64.1)	193(68)	1.00	0.344
	(rs1800871)	C	94(35.9)	91(32)	1.19(0.83-1.69)	
	-592	A	168(64.6)	194(68.8)	1.00	0.302
	(rs1800872)	C	92(35.4)	88(31.2)	1.21(0.84-1.73)	

Table 2. Genotypic and haplotypic distributions of *IL4* and *IL10* polymorphisms in patients in chronic HBV patients and healthy controls

Genes	SNPs	Genotype/haplotype	CHB n (%)	Control n (%)	OR (95% Confidence Interval)	*P*
TNFA	-308	AA	0(0)	2(1.4)	1.00	0.254
		AG	20(15.4)	30(21.3)	undefined	0.157
		GG	110(84.6)	109(77.3)	undefined	
	-238	AA	0(0)	0(0)	1.00	undefined
		AG	11(8.5)	8(5.6)	undefined	undefined
		GG	119(91.5)	134(94.4)	undefined	
	-308/238/	GG	229(88.1)	239(85.4)	1.03(0.80-1.33)	0.853
		AG	20(7.7)	33(11.8)	0.65(0.35-1.21)	0.192
		GA	11(4.2)	8(2.9)	1.48(0.54-4.10)	0.547
IL1A	-889	TT	1(0.8)	1(0.7)	1.00	0.970
		TC	19(14.6)	18(12.7)	1.06(0-42.46)	0.937
		CC	110(84.6)	123(86.6)	0.89(0.02-33.13)	
IL1B	-511	TT	31(23.8)	31(21.8)	1.00	0.569
		TC	63(48.5)	75(52.8)	0.84(0.44-1.60)	1.000
		CC	36(27.7)	36(25.4)	1.00(0.48-2.09)	
	+3962	TT	0(0)	0(0)	1.00	undefined
		TC	7(5.4)	4(2.8)	undefined	undefined
		CC	123(94.6)	138(97.2)	undefined	
IL1R	pst1 1970	TT	13(9.9)	18(12.7)	1.00	0.821
		TC	57(43.5)	72(50.7)	1.10(0.46-2.61)	0.235
		CC	61(46.6)	52(36.6)	1.62(0.68-3.92)	
IL1RA	mspa1 11100	CC	32(24.2)	36(25.3)	1.00	0.481
		CT	67(50.8)	61(43.0)	1.24(0.66-2.32)	0.564
		TT	33(25.0)	45(31.7)	0.82(0.41-1.67)	
IL6	-174	CC	0(0)	1(0.7)	1.00	0.236
		CG	5(3.8)	3(2.1)	undefined	0.340
		GG	126(96.2)	138(97.2)	undefined	
TGFB	Codon 10	TT	20(20.4)	21(17.2)	1.00	0.857
		TC	58(59.2)	65(53.3)	0.94(0.44-2.02)	0.197
		CC	20(20.4)	36(29.5)	0.58(0.24-1.44)	
	Codon 25	CC	0(0)	0(0)	1.00	undefined
		CG	2(1.7)	1(0.8)	undefined	undefined
		GG	119(98.3)	119(99.2)	undefined	
IL2	-330	GG	23(18.5)	19(13.6)	1.00	0.183
		GT	47(37.9)	63(45.0)	0.62(0.28-1.34)	0.369
		TT	54(43.6)	58(41.4)	0.77(0.36-1.66)	
	+166	TT	21(16.3)	24(16.9)	1.00	0.775
		TG	59(45.7)	61(43.0)	1.11(0.53-2.32)	0.960
		GG	49(38.0)	57(40.1)	0.98(0.46-2.10)	
IFNG	+874	TT	12(9.2)	14(9.9)	1.00	0.918
		TA	45(34.6)	50(35.5)	1.05(0.40-2.73)	0.813
		AA	73(56.2)	77(54.6)	1.11(0.45-2.76)	
IL12	-1188	CC	29(22.3)	28(19.9)	1.00	0.805
		CA	69(53.1)	72(51.0)	0.93(0.48-1.79)	0.425
		AA	32(24.6)	41(29.1)	0.75(0.35-1.60)	
IL18	-137	CC	4(3.1)	1(0.7)	1.00	0.355
		GC	28(21.5)	31(21.8)	0.23(0.01-2.39)	0.196
		GG	98(75.4)	110(77.5)	0.22(0.01-2.16)	

Table 2. Continued

Genes	SNPs	Genotype/haplotype	CHB n (%)	Control n (%)	OR (95% Confidence Interval)	*P*
IL4	-1098	GG	1 (0.8)	0 (0)	1.00	0.358
		GT	22 (16.9)	19 (13.4)	0.00(0-21.89)	0.285
		TT	107 (82.3)	123 (86.6)	0.00(0-15.29)	
	-590	TT	83(63.8)	75(52.8)	1.00	**0.063**
		CT	38(29.2)	56(39.4)	0.61(0.35-1.06)	0.525
		CC	9(7.0)	11(7.8)	0.74(0.26-2.05)	
	-33	CC	9(6.9)	11(7.8)	1.00	0.860
		CT	42(32.1)	56(39.4)	0.92(0.32-2.68)	0.578
		TT	80(61.0)	75(52.8)	1.30(0.47-3.66)	
	-1098/-590/-33	TCC	32(12.4)	60(21.1)	0.53(0.32-0.86)	**0.005**
		TTT	198(76.7)	204(71.8)	1.07(0.82-1.39)	0.663
		GCC	23(8.9)	18(6.3)	1.41(0.71-2.79)	0.375
IL4RA	+1902	GG	8(6.2)	8(5.8)	1.00	0.857
		GA	49(37.7)	54(38.8)	0.91(0.28-2.92)	0.919
		AA	73(56.1)	77(55.4)	0.95(0.30-2.96)	
IL10	-1082	GG	1(0.8)	0(0.0)	1.00	0.263
		GA	13(10.0)	17(12.0)	0(0-14.94)	0.300
		AA	116(89.2)	125(88.0)	0(0-16.30)	
	-819	TT	47(35.9)	67(47.2)	1.00	**0.024**
		CT	74(56.5)	59(41.5)	1.79(1.04-3.06)	0.796
		CC	10(7.6)	16(11.3)	0.89(0.34-2.31)	
	-592	AA	48(36.9)	68(48.2)	1.00	**0.028**
		CA	72(55.4)	58(41.1)	1.76(1.03-3.01)	0.899
		CC	10(7.7)	15(10.7)	0.94(0.36-2.47)	
	-1082/-819/-592	ATA	167(64.2)	191(67.7)	0.95(0.72-1.25)	0.748
		ACC	77(29.6)	70(24.8)	1.19(0.82-1.75)	0.392
		GCC	15(5.8)	17(6.0)	0.96(0.44-2.06)	0.951
		ACA	1(0.4)	3(1.1)	0.36(0.01-3.90)	0.679

- ***IL6***

Regarding the position *IL6* -174 where the GG genotype represented the majority of the population,[21] chronic hepatitis patients in Italy were reported to have a higher frequency of G allele than HBcAb negative controls (OR =1.484, CI =0.975-2.260, p <0.05)[22] and a similar result was observed in a study in Brazil.[16] However, we did not observe any positive association in our study.

Anti-inflammatory cytokines

- ***TGFB***

As for *TGFB*, no statistically significant difference in the genetic ability to produce TGF- β between the HBV group and the controls has been previously observed.[21] In the same vein, we did not observe any positive association in this study.

Th1 cytokines

- ***IL2***

The *IL2* -330T allele and TT genotype were associated with an increased risk of persistent HBV (p =0.03, OR =7.14 and p =0.01, OR =2.26) in the Chinese population,[23] and the risk of progression and a chronic course of viral hepatitis in Caucasian populations has also been linked with T allele in *IL2*.[24] By contrast, we did not observe any positive association in our study.

IFNG

Lower *IL4* expression AA genotype is more often found in Asians, who are more susceptible to HBV than Caucasians.[25] The *IFNG* +874 AA genotype or A allele were reported to be associated with an increased risk.[21,23,26] Nevertheless, in this study, we could not find any significant association

Table 3. Distributions of T-helper cytokine genotypes in chronic HBV patients and healthy controls

Cytokine genotype	No. of cases	No. of controls	OR (95% Confidence Interval)	*p-value*
Th1 genes				
***IFNG* (+874)**				
TT	12	14	1.00	
AT/AA[a]	118	127	1.08 (0.45-2.62)	0.845
***IL12* (-1188)**				
AA	32	41	1.00	
AC/CC[a]	98	100	1.26 (0.71-2.23)	0.408
***IL18* (-137)**				
GG	98	110	1.00	
GC/CC[a]	32	32	1.12 (0.62-2.04)	0.686
Total low-activity Th1 genotypes[b]				
0	3	3	1.00	
1	30	39	0.77 (0.11-5.24)	0.758
2	73	76	0.96 (0.15-6.20)	0.961
3	24	22	1.09 (0.15-7.80)	0.920
Th2 genes				
***IL4* (-590)**				
CT/TT	121	131	1.00	
CC[a]	9	11	0.89 (0.32-2.40)	0.795
***IL10* (-1082 and -819)**				
GG/AG and CC/CT	14	17	1.00	
AA and/or TT[a]	116	125	1.13 (0.50-2.54)	0.755
Total low-activity Th2 genotypes[c]				
0	14	14	1.00	
1	106	120	0.88 (0.38-2.07)	0.757
2	9	8	1.13 (0.28-4.46)	0.848

[a]Putative low-activity genotypes.

[b]Summed across *IFNG, IL12 and IL18* genotypes.

[c]Summed across *IL4 and IL10* genotypes.

between polymorphisms of +874 and HBV infection in accordance with several of the previously reported studies.[16,27]

IL12 and *IL18*

There are limited studies on *IL12* polymorphism with chronic HBV. *IL18* -137C was reported to be a protective allele.[28] Additionally, two studies have reported that -607A was a protective allele.[29,30] Here, we did not find any positive association with *IL12* and *IL18* polymorphism as investigated in this study.

Th2 cytokines

- *IL4*

One study in the Chinese population reported *IL4*-590 polymorphism was not associated with susceptibility to chronic hepatitis B.[23] However, the TT genotype of *IL4* -590 was associated with the risk of progression and chronic course of hepatitis in the Caucasian population.[24] In our study, we observed a trend suggesting that -590 TT could be a risk genotype. Particularly, our finding substantiated the importance of the -1098/-590/-33 TCC as a protective haplotype for chronic hepatitis B in the Thai population. We hypothesize that the low activity of *IL4* gene promoter resulted in a lower level of Th2 cytokine. A lower suppression of IFN-γ production could thus be a major protective genetic factor for chronic hepatitis B infection in Thai population.

- *IL10*

Studies of the effects of *IL10* on HBV infection have also been inconclusive. According to a meta-analysis of approximately 1,500 chronically infected patients and 1,300 controls at position -1082G/A, when compared to GA+GG, AA genotype was reported to be protective of HBV infection (OR =0.684, CI =0.476-0.982, p =0.04).[31] As for the position -592A/C, when compared to AA, AC genotype was reported to be a risk factor for HBV infection (OR = 1.343, 95% CI = 1.017-1.684, P = 0.011).[31] Similarly, in another study in the Chinese population, the frequency of the AA genotype at position -592A/C was significantly lower in chronic HBV patients (OR =0.67, CI =0.51-0.94, P =0.018).[32] The haplotype ACC of immediate level of IL-10 production was closely associated with chronic liver disease (p =0.004), whereas haplotype ATA and homozygous ATA/ATA (low level of IL-10) were associated with protection (p =0.035).[33]

In our study, we found a similar trend. When compared to -819 TT and -592 AA, -819 CT and -592 CA were associated with a risk of developing chronic hepatitis B. It should be noted that -819 and -592 polymorphisms in the *IL10* gene were in a complete linkage. Possibly due to the small sample sizes, we did not observe any statistically significant association between disease risks with the ACC haplotype. Interestingly, IL-10 is not produced only from Th2 cells; another major source isregulatory cells. It has a regulatory function to down-regulate HBV specific CD8 T cell response.[34] However, the genetic association result consistently reported is

Table 4. The combined effect of T-helper1 and T-helper2 genotypes on the risk of chronic HBV

	Total no. of low activity Th2 genotypes[a]					
	1 or 2			0		
Total no. of low activity Th1 genotypes[b]	**Cases/ controls**	**OR (95% CI)**	***p-value***	**Cases/ controls**	**OR (95% CI)**	***p-value***
0	3/3	1.00		0/0	Undefined	
1	27/35	0.77(0.11-5.31)	0.761	3/4	0.75 (0.05-11.28)	0.797
2	63/69	0.91(0.14-5.93)	0.913	9/7	1.29 (0.14-12.19)	0.793
3	22/19	1.16(0.16-8.43)	0.867	2/3	0.67 (0.03-13.47)	0.740

that the patients with low IL-10 as well as low IL-4 producing allele have a lower risk of chronicity, suggesting a protective role of Th2 rather than an active regulatory role for the *IL10* gene.

- ***Role of the combination of Th1 and Th2 cytokines***

Cytotoxic T lymphocytes (CTLs) and Th1 cells are well known to play a central role in the control of viral infection including HBV infection and defects in their function lead to persistence of HBV infection.[4-5] One possible risk for chronic HBV development is host genetic factor causing low levels of Th1 or high levels of Th2 cytokine expression.[23,27,32] However, so far most of the previous studies have only analyzed each gene separately and there have been limited efforts to analyze a combination of cytokine genes, such as as Th1 and Th2. In this preliminary study, after analyzing the combination of Th1 and Th2 genotypes in the way that was performed in a study of genetic risk factor to HBV-related hepatocellular carcinoma[35], we did not observe any significant associations. A non-significant association among such combinations could have resulted from the limitation of our small sample size. However, reports of the genetic association results for individual genotypes and haplotypes consistently indicate that patients with low IL-10 as well as a low IL-4 producing allele have a lower risk for chronicity, suggesting a protective role of Th2. Further studies with more samples are needed to completely analyze the role of the Th1/Th2 combination in chronic hepatitis B infection.

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Conflict of interest

All authors report no conflicts of interest relevant to this article.

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