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Association of interferon-alpha gene polymorphisms with chronic hepatitis B virus infection

I. Kimkong*,†, P. Tangkijvanich‡ & N. Hirankarn§

Summary

In this study, the association between the risk of chronic hepatitis B virus infection and the polymorphisms within promoter regions of IFN-α1 and five genes was explored. This association study was performed on 180 Thai patients with chronic HBV infection [hepatocellular carcinoma (HCC) = 65 and non-HCC = 115], 173 individuals with self-limited HBV infection and 140 healthy controls. Our results showed that the A allele of -1823G/A SNP within *IFNA1* gene was significantly associated with an increased risk of chronic HBV infection as compared to healthy individuals and self-limited HBV group [OR (95% CI) = 2.20 (1.51–3.19), $P = 0.000014$ and OR (95% CI) = 1.61 (1.12–2.33), $P = 0.0073$, respectively]. The effect of A allele was similar to autosomal recessive in which the presence of AA genotype when compared to GG and GA conferred the OR of 2.79 (95% CI = 1.72–4.52, $P = 0.0000085$). By multifactor dimensionality reduction analysis, we found the interaction between *IFNA5* (-2529) and *IFNA1* (-1823) genes that gave the risk to chronic HBV infection, with the OR (95% CI) of the high-risk to low-risk group was 2.79 (1.77–4.40), $P < 0.0001$. However, further study in functional significance is required.

Introduction

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 become carriers of the virus. Some of them develop liver cirrhosis and hepatocellular carcinoma. Approximately one million people die each year from these complications (Lavanchy, 2004). Thailand has been classified as a region of intermediate endemicity as well as Taiwan, India, Pakistan, Korea and Philippines (Mohamed *et al.*, 2004). Twin studies in Chinese showed a higher concordance rate for HBeAg persistence in monozygotic twins compared with dizygotic twins (Lin *et al.*, 1989). This provides the strong evidence for a host genetic effect. Therefore, the difference of host genetic might affect the different outcomes of patients with HBV infection. Several association studies reported that various human leucocyte antigen (HLA) class I and II loci, many cytokine and cytokine receptor genes were associated with HBV susceptibility and/or HBV persistence or disease severity (Thursz *et al.*, 2011).

Interferon-alpha (IFN-α) is a cytokine that mainly involved in innate immune response against viral infection. The introduction of IFN-α by several stimuli has been shown to inhibit HBV gene expression and/or HBV replication in the livers of HBV-transgenic mice (Cavanaugh *et al.*, 1998). Furthermore, it has been widely used in the treatment of chronic HBV infection (Karayiannis, 2003). There are 13 subtypes in the IFN-α family that composed of *IFNA1*, *IFNA2*, *IFNA4*, *IFNA5*, *IFNA6*, *IFNA7*, *IFNA8*, *IFNA10*, *IFNA13*, *IFNA14*, *IFNA16*, *IFNA17 and IFNA21*. These genes are located in a cluster on the short arm of human chromosome 9 (Diaz *et al.*, 1994). *IFNA2* is a major subtype of IFNA species that is produced in response to many viruses. In addition, it is often used to treat hepatitis B in combination with the other antiviral drugs (Bonino *et al.*, 2007). An association study of *IFNA2* gene found that a deletion in the promoter was significantly more frequent in HBV-infected Vietnamese patients than in control individuals. Moreover, the deletion in promoter of *IFNA2* gene reduces the transcription of this gene *in vitro* (Song le *et al.*, 2006). However, there is no association study between HBV infection and the other major subtypes such as *IFNA1* and *IFNA5*, which are interesting subtypes in

* Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand, † Center for Advanced Studies in Tropical Natural Resources, National Research University-Kasetsart University, Kasetsart University, Bangkok, Thailand, ‡ Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand and § Immunology Unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

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Correspondence: Nattiya Hirankarn, Immunology Unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Rama 4 Road, Bangkok 10330, Thailand. Tel: +66 2 2564132; ext. 624; Fax: +66 2 2525959; E-mail: fmednpt@md.chula.ac.th

response to viral infection. *IFNA1* and *IFNA5* have been reported to be expressed in the liver tissue, especially *IFNA5* which is the predominant subtype (Castelruiz *et al.*, 1999). Furthermore, the expression of *IFNA5* is higher than other IFNA subtypes in peripheral blood mononuclear cell (PBMC) of patients infected with some viruses such as hepatitis C virus (Castelruiz *et al.*, 1999; Larrea *et al.*, 2001). Polymorphisms in the promoter region of these genes might influence such gene expression.

Therefore, we investigated the association between single nucleotide polymorphism (SNP) within the promoter region of *IFNA1* and *IFNA5* genes with susceptibility to chronic HBV infection and the development of HCC.

Materials and methods

Subjects

One hundred and eighty 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 HBsAg over a 6-month period and did not have any other type of liver diseases such as chronic hepatitis C or alcoholic liver disease. In addition, all the patients had elevated serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. Patients with chronic HBV infection were further divided into two groups: without ($N = 115$; 34 women and 81 men: mean age ± SD = 44.44 ± 14.63 years) and with HCC ($N = 65$; 10 women and 55 men: mean age ± SD = 53.96 ± 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^{-1}. Moreover, self-limited HBV group served as control for the population-based case–control study contained 173 subjects (91 women and 82 men: mean age ± SD = 48.41 ± 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, 140 ethnically and geographically matched controls (83 women and 57 men: mean age ± SD = 23.09 ± 12.58 years) from healthy blood donor of the Thai Red Cross Society were recruited as control group. The ethics committee of the faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, approved the study, and the subjects gave their informed consent.

Moreover, chronic HBV patients with AST and ALT level of ≥ 100 U L^{-1} were defined as high AST and ALT groups, while the patients with AST and ALT <100 U L^{-1} were classified as low liver enzyme groups. Patients with HBV DNA levels of $\geq 10^5$ copies per mL were characterized as high viral load group, whereas the patients with HBV DNA levels of $<10^5$copies per mL were considered as low viral replication subjects (Li *et al.*, 2011).

DNA extraction and genotyping study

DNA was extracted from the buffy coat collected with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, using a salting-out method (Miller *et al.*, 1988). DNA was aliquoted and stored at −20 °C until used. Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) was used to analyse the polymorphism of *IFNA1* gene (-1823G/A, rs1332190) and *IFNA5* gene (-2529A/T, rs3758236) as previously described (Hirankarn *et al.*, 2008). Negative controls without DNA template were included in each experiment. Ten per cent 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 and 95% confidence intervals, as well as for haplotype analysis (Purcell *et al.*, 2007). A P value of <0.05 was considered statistically significant. In addition, gene–gene interaction was analysed by multifactor dimensionality reduction (MDR) approach (Hahn *et al.*, 2003).

Results

The distribution of genotype and allele frequencies of *IFNA1* and *IFNA5* gene polymorphisms in chronic HBV patients (with HCC and without HCC), self-limited HBV patients and healthy controls are shown in Table 1. In this study, both SNPs were in Hardy–Weinberg equilibrium when compared the observed and expected genotype frequencies of each SNP ($P > 0.05$). In this study, we found statistically significant differences in the allele frequency of -1823G/A SNP (rs1332190) within *IFNA1* gene between patients with chronic HBV and healthy controls. The A allele of this SNP was significantly associated with an increased risk in chronic HBV infection as compared to healthy individuals [OR (95% CI) = 2.20 (1.51–3.19), $P = 0.000014$], whereas the comparison between patients with chronic HBV and self-limited HBV group showed lower statistical significance with the OR (95% CI) of 1.61 (1.12–2.33) and $P = 0.0073$. The effect of A allele was similar to autosomal recessive in which the presence of AA genotype when compared to GG and GA conferred the OR of

Table 1. Genotype and allele frequencies of *IFNA1* and *IFNA5* gene polymorphisms in chronic HBV patients (with HCC and without HCC), self-limited HBV patients and healthy controls

SNP	Genotype/allele	Total chronic HBV *N* = 180 (%)	With HCC *N* = 65 (%)	Without HCC *N* = 115 (%)	Self-limited HBV *N* = 173 (%)	Healthy control *N* = 140 (%)
IFNA1 (-1823) (rs1332190)	GG	7 (3.89)	2 (3.08)	5 (4.35)	16 (9.25)	13 (9.29)
	GA	56 (31.11)	20 (30.77)	36 (31.30)	65 (37.57)	71 (50.71)
	AA	117 (65.00)[b]	43 (66.15)[e]	74 (64.35)[h]	92 (53.18)	56 (40.00)
	G	70 (19.44)	24 (18.46)	46 (20.00)	97 (28.03)	97 (34.64)
	A	290 (80.56)[a,c]	106 (81.54)[d,f]	184 (80.00)[g,i]	249 (71.97)	183 (65.35)
IFNA5 (-2529) (rs3758236)	AA	46 (25.56)	15 (23.08)	31 (26.96)	37 (21.39)	37 (26.43)
	AT	83 (46.11)	30 (46.15)	53 (46.09)	92 (53.18)	64 (45.71)
	TT	51 (28.33)	20 (30.77)	31 (26.96)	44 (25.43)	39 (27.86)
	A	175 (48.61)	60 (46.15)	115 (50.00)	166 (47.98)	138 (49.29)
	T	185 (51.39)	70 (53.85)	115 (50.00)	180 (52.02)	142 (50.71)

HBV, hepatitis B virus; HCC, hepatocellular carcinoma; SNP, single nucleotide polymorphism.
[a]A compared with G allele (total chronic HBV vs. healthy control); OR (95%CI) = 2.20 (1.51–3.19), $P = 0.000014$; [b]Autosomal recessive model AA compared with GA + GG genotype (total chronic HBV vs. healthy control); OR (95%CI) = 2.79 (1.72–4.52), $P = 0.0000085$; [c]A compared with G allele (total chronic HBV vs. self-limited HBV); OR (95%CI) = 1.61 (1.12–2.33), $P = 0.0073$; [d]A compared with G allele (chronic HBV with HCC vs. healthy control); OR (95%CI) = 2.34 (1.37–4.02), $P = 0.00083$; [e]Autosomal recessive model AA compared with GA + GG genotype (chronic HBV with HCC vs. healthy control); OR (95%CI) = 2.93(1.52–5.69), $P = 0.00049$; [f]A compared with G allele (chronic HBV with HCC vs. self-limited HBV); OR (95%CI) = 1.72 (1.01–2.93), $P = 0.033$; [g]A compared with G allele (chronic HBV without HCC vs. healthy control); OR (95%CI) = 2.12 (1.39–3.25), $P = 0.00025$; [h]Autosomal recessive model AA compared with GA + GG genotype (chronic HBV without HCC vs. healthy control); OR (95%CI) = 2.71 (1.58–4.66), $P = 0.0001$; [i]A compared with G allele (chronic HBV without HCC vs. self-limited HBV); OR (95%CI) = 1.56 (1.03–2.37), $P = 0.029$.

2.79 (95% CI = 1.72–4.52, $P = 0.0000085$). In addition, we classified total chronic HBV as with HCC ($N = 65$) and without HCC ($N = 115$). Our finding still showed a significant difference of A allele of -1823G/A SNP (rs1332190) between chronic HBV patients with HCC and healthy controls as well as chronic HBV patients without HCC vs. healthy controls [OR (95%CI) = 2.34 (1.37–4.02), $P = 0.00083$ and OR (95%CI) = 2.12 (1.39–3.25), $P = 0.00025$, respectively]. When we compared both chronic HBV groups (with and without HCC) vs. self-limited HBV patients, this A allele frequency was also found to be significantly different [OR (95% CI) = 1.72 (1.01–2.93), $P = 0.033$ and OR (95% CI) = 1.56 (1.03–2.37), $P = 0.029$, respectively]. The effect of A allele seems to be autosomal recessive model with the OR of 2.93 (95% CI = 1.52–5.69, $P = 0.00049$). However, we did not find any significant associations of -2529A/T SNP in *IFNA5* gene between patients with chronic HBV and control groups ($P > 0.05$).

Furthermore, we performed haplotype analysis of these two SNPs (-2529A/T SNP in *IFNA5* and -1823G/A SNP in *IFNA1*). There were four haplotypes including AG, TG, AA and TA. To test the association of *IFNA* haplotype and disease development, we compared each tested haplotype with other two haplotypes between patients and controls. In this study, we found significant association between TA haplotype and risk to chronic HBV infection [total chronic HBV (OR = 1.69, $P = 0.0065$), with HCC (OR = 1.91, $P = 0.0091$) and without HCC (OR = 1.64, $P = 0.0314$)]. In contrast, TG was observed to be protective haplotype [total chronic HBV (OR = 0.331, $P = 0.0004$), with HCC (OR = 0.328, $P = 0.0112$) and without HCC (OR = 0.333, $P = 0.0022$)]. The results of haplotype analysis are shown in Table 2. These haplotype results were consistent with the additional analysis of gene–gene interaction using MDR approach. In this study, the combination of TT genotype of *IFNA5* gene with AA genotype of *IFNA1* gene

Table 2. Haplotype analysis for -2529A/T (rs3758236) of *IFNA5* gene and -1823G/A (rs1332190) of *IFNA1* gene

	Haplotype frequency					Total vs. Healthy control		With HCC vs. Healthy control		Without HCC vs. Healthy control	
Haplotype	Total chronic HBV	With HCC	Without HCC	Self-limited HBV	Healthy control	OR	*P* value	OR	*P* value	OR	*P* value
AG	0.0973	0.0856	0.0999	0.1328	0.1513	0.579	0.0378	0.473	0.0838	0.631	0.1048
TG	0.0972	0.0990	0.1001	0.1475	0.1952	0.331	0.0004	0.328	0.0112	0.333	0.0022
AA	0.3888	0.3759	0.4001	0.3469	0.3416	1.230	0.2192	1.150	0.5565	1.280	0.1997
TA	0.4167	0.4394	0.3999	0.3727	0.3120	1.690	0.0065	1.910	0.0091	1.640	0.0314

HBV, hepatitis B Virus; HCC, hepatocellular carcinoma; OR, odds ratio.

Table 3. Analysis of gene–gene interaction using MDR approach for -2529A/T (rs3758236) of *IFNA5* gene and -1823G/A (rs1332190) of *IFNA1* gene

Combination	Chronic HBV	Healthy control	Case/control ratio	Predicted class
TT, AA	34	9	3.7778	High risk
AT, AA	53	28	1.8929	High risk
AA, AA	30	19	1.5789	High risk
TT, GA	16	26	0.6154	Low risk
AT, GA	28	33	0.8485	Low risk
AA, GA	12	12	1	Low risk
TT, GG	1	4	0.25	Low risk
AT, GG	2	3	0.6667	Low risk
AA, GG	4	6	0.6667	Low risk

MDR, multifactor dimensionality reduction.

(TT, AA) was classified as high-risk group (ratio of case: control >1) as well as AT, AA and AA, AA. Whereas the combination between TT and GA, AT and GA, AA and GA, TT and GG, AT and GG, AA and GG of *IFNA5* and *IFNA1* genes, respectively, was classified as low-risk group (ratio of case: control $\leq$ 1) (Table 3). By whole statistical analysis, the OR (95% CI) for the high-risk to low-risk group was 2.79 (1.77–4.40), $P < 0.0001$.

In addition, the association between allele, genotype and haplotype frequencies of *IFNA1* and *IFNA5* gene polymorphisms with HBV viral load and liver enzymes in a subset of the patients was analysed. In this study, there was no significant association between these gene polymorphisms with the level of HBV DNA, AST and ALT liver enzymes (Table 4).

Discussion

In this study, we determined the association between the *IFNA* gene polymorphisms and the susceptibility to chronic HBV infection. We focused on two polymorphisms in the promoter region comprising -1823G/A SNP (rs1332190) within *IFNA1* gene and -2529A/T SNP (rs3758236) within *IFNA5* gene. Our results suggest that *IFN* polymorphism might be important for the risk of chronic HBV infection. In this study, we found that A allele of -1823G/A SNP within *IFNA1* had a higher risk of developing chronic hepatitis B. By model of inheritance analysis, the association of this SNP seems to be recessive effect model. In addition, we divided chronic HBV patients into patient with HCC and without HCC groups. Our results showed that SNP within *IFNA1* was associated with both groups. This suggests that there may be other genetic

Table 4. Allele, genotype and haplotype frequencies in chronic hepatitis B patients stratified by HBV DNA levels, aspartate aminotransferase and alanine aminotransferase

SNPs	HVL *N* = 40 (%)	LVL *N* = 19 (%)	OR	*P* value	HAST *N* = 42 (%)	LAST *N* = 74 (%)	OR	*P* value	HALT *N* = 45 (%)	LALT *N* = 72 (%)	OR	*P* value
Alleles												
IFNA1												
rs1332190												
G	17 (21.25)	4 (10.53)	2.29	0.1547	18 (21.43)	30 (20.27)	1.07	0.8342	21 (23.33)	28 (19.44)	1.26	0.4769
A	63 (78.75)	34 (89.47)			66 (78.57)	118 (79.73)			69 (76.67)	116 (80.56)		
IFNA5												
rs3758236												
A	41 (51.25)	17 (44.74)	1.3	0.5084	42 (50.00)	72 (48.65)	1.06	0.8431	44 (48.89)	70 (48.61)	1.01	0.967
T	39 (48.75)	21 (55.26)			42 (50.00)	76 (51.35)			46 (51.11)	74 (51.39)		
Genotypes												
IFNA1												
rs1332190												
GG	1 (2.50)	0 (0)	1	1	2 (4.76)	2 (2.70)	1.8	0.5591	2 (4.44)	2 (2.78)	1.63	0.638
GA	15 (37.50)	4 (21.05)	2.25	0.2065	14 (33.33)	26 (35.14)	0.92	0.8444	17 (37.78)	24 (33.33)	1.21	0.624
AA	24 (60.00)	15 (78.95)	0.4	0.1508	26 (61.91)	46 (62.16)	0.99	0.9781	26 (57.78)	46 (63.89)	0.77	0.5086
IFNA5												
rs3758236												
AA	11 (27.50)	4 (21.05)	1.42	0.5951	11 (26.19)	18 (24.32)	1.1	0.8235	12 (26.67)	17 (23.61)	1.18	0.7096
AT	19 (47.50)	9 (47.37)	1.01	0.9925	20 (47.62)	36 (48.65)	0.96	0.9151	20 (45.00)	36 (50.00)	0.8	0.5584
TT	10 (25.00)	6 (31.58)	0.72	0.5953	11 (26.19)	20 (27.03)	0.96	0.922	13 (28.89)	19 (26.39)	1.13	0.7679
Haplotypes												
AG	0.1153	0.0417	4.74	0.1952	0.1328	0.1218	1.13	0.8076	0.129	0.1188	1.12	0.8166
TG	0.0972	0.0636	2.14	0.5435	0.0815	0.0809	1.01	0.9882	0.1043	0.0757	1.76	0.4484
AA	0.3972	0.4057	0.97	0.9299	0.3647	0.3647	1.01	0.9699	0.3599	0.3673	0.97	0.9083
TA	0.3903	0.4891	0.65	0.3102	0.4326	0.4326	0.94	0.8351	0.4068	0.4382	0.87	0.6361

HBV, hepatitis B virus; SNPs, single nucleotide polymorphisms; HVL, high viral load, HBV DNA level $\geq 10^5$ copies per ml; LVL, low viral loads, HBV DNA level $<10^5$ copies per ml; HAST, high aspartate aminotransferase, AST level $\geq$100 U L^{-1}; LAST, low aspartate aminotransferase, AST level <100 U L^{-1}; HALT, high alanine aminotransferase, ALT level $\geq$100 U L^{-1}; LALT, low alanine aminotransferase, ALT level <100 U L^{-1}; OR, odds ratio.

factors responsible for the development of HCC. Recent studies reported that some cytokine polymorphisms such as SNPs in *IL-1B*, *TNF-α* and *IL-28B* affected the progression of HCC (Hirankarn *et al.*, 2006; Kummee *et al.*, 2007; Ren *et al.*, 2012). Our significant finding was not seen between -2529A/T SNP in *IFNA5* gene and any groups of patients with chronic HBV. However, haplotype analysis revealed significant association between haplotype TA and risk to chronic HBV infection. This result corresponded to the analysis of genotype combination using MDR program (Hahn *et al.*, 2003). The genotype having two T allele of *IFNA5* gene and two A allele of *IFNA1* gene had higher risk effect than the other combinations. This is consistent to our previous study in *IFNA1* and *IFNA5* genes interaction (Hirankarn *et al.*, 2008).

IFN-α is widely used in patients infected with HBV to provide the lack of this cytokine. However, there are no data for different patterns of IFNA subtypes that produced in patients with HBV. The activity of IFN-α is mediated by type I interferon receptor, which composed of IFNAR1 and IFNAR2 (Pestka *et al.*, 2004). A mechanism to control the level of IFN-α was studied by *in vitro* analysis of the promoter variants in *IFNA2* gene (Song le *et al.*, 2006). For *IFNA1* and *IFNA5* genes, our previous report showed that these two genes were associated with some diseases such as systemic lupus erythematosus (SLE) (Hirankarn *et al.*, 2008). However, there were no any previous reports in association between polymorphisms of these two genes and HBV infection. There was only one report in type I interferon receptor genes such as *IFNAR1* and *IFNAR2*. Several studies found that polymorphisms in promoter, intron, exon and 3′UTR regions of *IFNAR1* gene were associated with HBV infection (Zhou *et al.*, 2007; Song *et al.*, 2008; Zhou *et al.*, 2009; He *et al.*, 2010; Zhou *et al.*, 2012). For *IFNAR2* gene, a study in polymorphism of this gene showed that non-synonymous SNP located on signal peptide region was associated with outcome of persistent HBV infection in Gambians (Frodsham *et al.*, 2006). However, this association was not observed in Chinese patients (Chen *et al.*, 2010).

In conclusion, our finding showed the association between polymorphisms in promoter region of *IFNA1* and *IFNA5* genes and the risk to chronic HBV infection. However, until now, there is still no any report in functional significance of polymorphisms in these two genes. Thus, functional analysis involving with the effect of promoter polymorphism on the gene expression is required in the future.

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References

Bonino, F., Marcellin, P., Lau, G.K., Hadziyannis, S., Jin, R., Piratvisuth, T. *et al.* (2007) Predicting response to peginterferon alpha-2a, lamivudine and the two combined for HBeAg-negative chronic hepatitis B. *Gut*, **56**, 699.

Castelruiz, Y., Larrea, E., Boya, P., Civeira, M.P. & Prieto, J. (1999) Interferon alfa subtypes and levels of type I interferons in the liver and peripheral mononuclear cells in patients with chronic hepatitis C and controls. *Hepatology*, **29**, 1900.

Cavanaugh, V.J., Guidotti, L.G. & Chisari, F.V. (1998) Inhibition of hepatitis B virus replication during adenovirus and cytomegalovirus infections in transgenic mice. *Journal of Virology*, **72**, 2630.

Chen, D.Q., Zeng, Y., Zhou, J., Yang, L., Jiang, S., Huang, J.D., Lu, L. & Zheng, B.J. (2010) Association of candidate susceptible loci with chronic infection with hepatitis B virus in a Chinese population. *Journal of Medical Virology*, **82**, 371.

Diaz, M.O., Pomykala, H.M., Bohlander, S.K., Maltepe, E., Malik, K., Brownstein, B. & Olopade, O.I. (1994) Structure of the human type-I interferon gene cluster determined from a YAC clone contig. *Genomics*, **22**, 540.

Frodsham, A.J., Zhang, L., Dumpis, U., Taib, N.A., Best, S., Durham, A. *et al.* (2006) Class II cytokine receptor gene cluster is a major locus for hepatitis B persistence. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 9148.

Hahn, L.W., Ritchie, M.D. & Moore, J.H. (2003) Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. *Bioinformatics*, **19**, 376.

He, X.X., Chang, Y., Jiang, H.J., Tang, F., Meng, F.Y., Xie, Q.H., Li, P.Y., Song, Y.H. & Lin, J.S. (2010) Persistent effect of IFNAR-1 genetic polymorphism on the long-term pathogenesis of chronic HBV infection. *Viral Immunology*, **23**, 251.

Hirankarn, N., Kimkong, I., Kummee, P., Tangkijvanich, P. & Poovorawan, Y. (2006) Interleukin-1beta gene polymorphism associated with hepatocellular carcinoma in hepatitis B virus infection. *World Journal of Gastroenterology*, **12**, 776.

Hirankarn, N., Tangwattanachuleeporn, M., Wongpiyabovorn, J., Wongchinsri, J. & Avihingsanon, Y. (2008) Genetic association of interferon-alpha subtypes 1, 2 and 5 in systemic lupus erythematosus. *Tissue Antigens*, **72**, 588.

Karayiannis, P. (2003) Hepatitis B virus: old, new and future approaches to antiviral treatment. *Journal of Antimicrobial Chemotherapy*, **51**, 761.

Kummee, P., Tangkijvanich, P., Poovorawan, Y. & Hirankarn, N. (2007) Association of HLA- DRB1*13 and TNF-alpha gene polymorphisms with clearance of chronic hepatitis B infection and risk of hepatocellular carcinoma in Thai population. *Journal of Viral Hepatitis*, **14**, 841.

Larrea, E., Alberdi, A., Castelruiz, Y., Boya, P., Civeira, M.P. & Prieto, J. (2001) Expression of interferon-alpha subtypes in peripheral mononuclear cells from patients with chronic hepatitis C: a role for interferon-alpha5. *Journal of Viral Hepatitis*, **8**, 103.

Lavanchy, D. (2004) Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *Journal of Viral Hepatitis*, **11**, 97.

Li, W., Jiang, Y., Jin, Q., Shi, X., Jin, J., Gao, Y., Pan, Y., Zhang, H., Jiang, J. & Niu, J. (2011) Expression and gene

polymorphisms of interleukin 28B and hepatitis B virus infection in a Chinese Han population. *Liver International*, **31**, 1118.

Lin, T.M., Chen, C.J., Wu, M.M., Yang, C.S., Chen, J.S., Lin, C.C., Kwang, T.Y., Hsu, S.T., Lin, S.Y. & Hsu, L.C. (1989) Hepatitis B virus markers in Chinese twins. *Anticancer Research*, **9**, 737.

Miller, S.A., Dykes, D.D. & Polesky, H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, **16**, 1215.

Mohamed, R., Desmond, P., Suh, D.J., Amarapurkar, D., Gane, E., Guangbi, Y. *et al.* (2004) Practical difficulties in the management of hepatitis B in the Asia-Pacific region. *Journal of Gastroenterology and Hepatology*, **19**, 958.

Pestka, S., Krause, C.D. & Walter, M.R. (2004) Interferons, interferon-like cytokines, and their receptors. *Immunological Reviews*, **202**, 8.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D. *et al.* (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, **81**, 559.

Ren, S., Lu, J., Du, X., Huang, Y., Ma, L., Huo, H., Chen, X. & Wei, L. (2012) Genetic variation in IL28B is associated with the development of hepatitis B-related hepatocellular carcinoma. *Cancer Immunology Immunotherapy*, **61**, 1433.

Song le, H., Toan, N.L., Xuan, N.T., Uhlemann, A.C., Boldt, A.B., Duy, D.N., Binh, V.Q., Kremsner, P.G. & Kun, J.F. (2006) A promoter polymorphism in the interferon alpha-2 gene is associated with the clinical presentation of hepatitis B. *Mutation Research*, **601**, 137.

Song le, H., Xuan, N.T., Toan, N.L., Binh, V.Q., Boldt, A.B., Kremsner, P.G. & Kun, J.F. (2008) Association of two variants of the interferon-alpha receptor-1 gene with the presentation of hepatitis B virus infection. *European Cytokine Network*, **19**, 204.

Thursz, M., Yee, L. & Khakoo, S. (2011) Understanding the host genetics of chronic hepatitis B and C. *Seminars in Liver Disease*, **31**, 115.

Zhou, J., Lu, L., Yuen, M.F., Lam, T.W., Chung, C.P., Lam, C.L. *et al.* (2007) Polymorphisms of type I interferon receptor 1 promoter and their effects on chronic hepatitis B virus infection. *Journal of Hepatology*, **46**, 198.

Zhou, J., Smith, D.K., Lu, L., Poon, V.K., Ng, F., Chen, D.Q., Huang, J.D., Yuen, K.Y., Cao, K.Y. & Zheng, B.J. (2009) A non-synonymous single nucleotide polymorphism in IFNAR1 affects susceptibility to chronic hepatitis B virus infection. *Journal of Viral Hepatitis*, **16**, 45.

Zhou, C., Yu, Q., Chen, L., Wang, J., Zheng, S. & Zhang, J. (2012) A miR-1231 binding site polymorphism in the 3′UTR of IFNAR1 is associated with hepatocellular carcinoma susceptibility. *Gene*, **507**, 95.