A Common *HLA–DPA1* Variant is a Major Determinant of Hepatitis B Virus Clearance in Han Chinese

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A recent genome-wide study showed that the single nucleotide polymorphisms (SNPs) in the *HLA-DP* region were associated with chronic hepatitis B virus (HBV) infection in Japanese and Thai persons. We tested the effects of *HLA-DP* SNPs for all major HBV outcomes in Han Chinese ($n = 1742$): HBV resistance, clearance, chronic infection, cirrhosis, and hepatocellular carcinoma. *HLA - DPA1* rs3077 T was strongly associated with decreased risk of chronic HBV infection (odds ratio, .62; $P = .001$), consistent with the previous report. We showed for the first time to our knowledge that it is a predictor for HBV clearance (odds ratio, 2.41; $P < .001$). However, rs3077 was not associated with the development of cirrhosis or hepatocellular carcinoma.

Hepatitis B virus (HBV) infection is a significant public health problem. The World Health Organization estimates that 2 billion persons have been infected with HBV and that 350 million persons live with chronic HBV infection. Approximately 25% of persons with chronic HBV infection will develop cirrhosis and hepatocellular carcinoma (HCC) [1]. HBV infection rates are disparate among world populations with high prevalence rates recorded in East Asia and Africa; hepatitis B surface antigen (HBsAg) carrier rates are reported to be 5.3%–12% in China, 8% in Thailand, and ~10% in Africa [2]. HBV infection in Asian populations is maintained through mother-to-infant vertical transmission or early childhood infections. Approximately 90% of infants and preschool children with HBV infection will fail to achieve viral clearance and develop chronic HBV infection, whereas only 5%–10% of adult HBV infections lead to persistent infection [2]. A very small proportion of patients with persistent HBV infection can spontaneously clear the virus without treatment. It has been reported that, in western countries, 1%–2% of HBV carriers become HBsAg negative each year [3], whereas in populations where HBV infection is endemic, such as Han Chinese, the rate of HBsAg clearance is much lower (.05%–.1% per year) [4, 5].

Variable outcomes of HBV infection are likely to be multifactorial, with environmental, viral, and host genetic factors contributing to the observed variability in HBV clearance and pathogenesis. Candidate gene association studies have implicated a number of genes in HBV resolution or persistence, including *HLA* class I and II alleles [6] and non-*HLA* genes (eg, *MBP* [7]). *HLA - DRB1**1302 was consistently associated with HBV clearance in Gambian [8] and European American populations [6].

Recently, Kamatani, et al [9] performed the first genome-wide association study for chronic HBV infection with use of a 2-stage design with 786 Japanese with chronic hepatitis B carriers and 2201 control subjects. The group identified 11 correlated SNPs in the region of *HLA - DPA1* and *HLA - DPB1* and validated 2

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independent SNPs in 3 additional Japanese and Thai populations comprising >3000 patients and control subjects [9]. This study identified association of *HLA - DPA1* SNPs rs3077 and *HLA - DPB1* rs9277535 with chronic hepatitis infection, but they did not determine whether the association with persistent infection was attributable to clearance of HBV or resistance to HBV infection.

Because prevalence rates of HBV infection in China are extremely high, we established a population-based study to investigate host genetic factors associated with HBV infection and pathogenesis in the Chinese Han population from northern China [10]. In this report, we determined the effects of *HLA-DP* SNPs on HBV infection, clearance, and progression to cirrhosis and HCC in Han Chinese [10].

PATIENTS AND METHODS

Multicenter Chinese HBV Cohort

The Chinese HBV Cohort enrolled participants during 2003–2007 from hospitals in cities in northern China. An Internal Review Board at National Cancer Institute approved the study. Local internal review board approvals from participating hospitals and informed consent from each participant were obtained.

The case-control study comprises the full spectrum of HBV infection status: HBV case groups include natural clearance, chronic asymptomatic, and symptomatic HBV infection; cirrhosis; and HCC plus a group of hypernormal control subjects lacking serological evidence of previous or current HBV infection. Case definitions are in accordance with the predefined criteria [10], based on diagnosis protocol issued by the Association of Infectious Diseases and Parasites Diseases of China [11]. A total of 1742 samples were genotyped and analyzed in this study (Table 1). All individuals, except patients with HCC, were at least 40 years of age at enrollment, to allow for sufficient time of disease progression.

HBV clearance was defined for persons who had (1) tests seropositive for antibody to HBsAg (anti-HBs) and antibody to hepatitis B core antigen (anti-HBc) without the presence of HBsAg or (2) anti-HBs positive and no self-reported and clinic/hospital record of hepatitis B vaccination. All clearance participants were at least 40 years of age at enrollment, to avoid potential confounding by HBV vaccination that became available in the mid-1980s; infant vaccination was introduced in 1992 [12]. Hypernormal control subjects were at least 40 years of age at enrollment and were seronegative for any HBV serological markers and anti-HCV at enrollment. Chronic HBV infection was defined by 2 positive test results at least 1 year apart for HBsAg and anti-HBc and with no evidence of cirrhosis by imaging and laboratory tests. We used the term "hypernormal" to distinguish from the general "normal" population, which has a high prevalence of anti-HBc. All participants in this study were negative for hepatitis A virus, hepatitis D virus, and hepatitis E virus infection. Virological testing and criteria of cirrhosis and HCC are presented in the Supplementary Methods.

Table 1. Characteristics of Participants in the Hepatitis B Virus (HBV) Cohort

Characteristic	No.	Female, %	Age, mean± SD, years
Clearance	287	30.0	50 ± 9.6
Chronic hepatitis	591	37.0	46.0 ± 7.0
Liver cirrhosis	370	40.0	49.9 ± 9.0
Hepatocellular carcinoma	265	36.2	51.4 ± 10.4
HBV-negative control subject[a]	229	38.3	48.7 ± 7.3

NOTE. Abbreviations: SD, standard deviation.

[a] All HBV serological markers were negative.

SNP Genotyping

Two SNPs, *HLA - DPA1* rs3077 and *HLA - DPB1* rs9277535, were genotyped using TaqMan assays (Applied Bisosystems). The rs3077 C/T (major/minor alleles in Asians) in this report corresponds to the G/A alleles, respectively, on the reverse strand in Kamatani et al [9].

Statistical Analysis

We used SAS, version 9.13 (SAS Institute) for analyses. The logistic regression model was applied to case-control comparisons for the different phenotypic outcomes for dominant and additive genetic models, adjusted for sex and age. All *P* values are 2-sided.

RESULTS

To determine whether the *HLA - DPA1* and *HLA - DPB1* SNPs were associated with chronic hepatitis B in Han Chinese, we compared genotype frequencies between the chronically HBV -infected groups (ie, chronic HBV infection, cirrhosis, and HCC) and hypernormal control subjects lacking infection of multiple hepatitis viruses.

HLA-DPAI rs3077 and HBV Infection

Carriage of the *HLA - DPA1* rs3077 T allele was a protective factor for chronic HBV infection (odds ratio [OR], .62; 95% confidence interval [CI], .47–.83; adjusted $P = .001$; dominant model); the results were similarly significant for the additive model. Adjusting for age and sex did not change the results (Table 2). This result replicates and extends the Kamatani et al [9] findings of the rs3077 association with persistent HBV infection in Japanese and Thai Asians to Han Chinese.

HLA-DPA1 rs3077 and HBV Clearance

We next determined whether *HLA - DPA1 rs3077* was associated with HBV clearance, a phenotype not explored by the genome-

Table 2. Association of *HLA - DPA1* Variant rs3077 With Hepatitis B Virus (HBV) Outcomes

	Cases, *n* (%)			Controls, *n* (%)				Dominant (T allele)[a]		Additive (T allele)[a]	
Phenotype	TT[b]	TC	CC	TT	TC	CC		OR (95% CI)	*P*	OR (95% CI)	*P*
	HBV chronic infection (*n* = 1,218)			**Hypernormal individuals[c] (*n* = 227)**							
HBV susceptibility	113 (.09)	485 (.40)	620 (.51)	35 (.15)	103 (.45)	89 (.39)	unadj.	.62 (.47–.83)	.0013	.68 (.55–.84)	.0002
							adj.	.62 (.47–.83)	.0013	.68 (.55–.84)	.0002
	Clearance (*n* = 287)			**HBV chronic infection[d] (*n* = 1,218)**							
HBV Clearance	41 (.14)	160 (.56)	86 (.30)	113 (.09)	485 (.40)	620 (.51)	unadj.	2.42 (1.84–3.20)	3.47×10^{-10}	1.77 (1.47–2.14)	3.75×10^{-9}
							adj.	2.41 (1.83–3.18)	4.65×10^{-10}	1.77 (1.46–2.14)	4.31×10^{-9}
	Cirrhosis (*n* = 370)			**Chronic hepatitis B (*n* = 591)**							
Cirrhosis	31 (.08)	143 (.39)	196 (.53)	61 (.10)	241 (.41)	289 (.49)	unadj.	.85 (.66–1.10)	.22	.87 (.71–1.06)	.17
							adj.	.84 (.64–1.10)	.2	.86 (.70–1.05)	.15
	HCC (*n* = 265)			**All other HBV chronic infection[e] (*n* = 961)**							
HCC	23 (.09)	104 (.39)	138 (.52)	92 (.10)	384 (.40)	485 (.50)	unadj.	.94 (.71–1.23)	.64	.94 (.77–1.16)	.58
							adj.	.93 (.70–1.23)	.6	.93 (.75–1.15)	.52

NOTE. CI, confidence interval; HCC, hepatocellular carcinoma; OR, odds ratio.

[a] Analyses were done using logistic regression model, with adjustment (adj.) of age groups (<50 or ⩾50 years) and sex, or without adjustment (unadj.).

[b] The 'T' or 'C' allele corresponds to 'A' or 'G' allele, respectively, in Kamatani et al [9], which were referred to the reverse strand of the sequence.

[c] Individuals without any HBV serological markers.

[d] Includes chronic HBV infection, cirrhosis and HCC.

[e] Includes chronic HBV infection and cirrhosis.

wide association study [9]. We compared a group of 287 individuals who spontaneously cleared HBV infection with 1216 individuals with chronic HBV infection. The rs3077 T allele was strongly predictive of HBV clearance in the dominant model (OR, 2.41; 95% CI, 1.83–3.18; $P < .001$), with similar effects for the additive model (OR, 1.77; adjusted $P < .001$) (Table 2). Results were very similar with and without adjustment for sex and age. We separately tested for association with the group with only anti-HBs reflecting full resolution of HBV ($n = 105$) or with the group with both anti-HBs and anti-HBc reflecting partial HBV clearance; results in both clearance groups remained highly significant (Supplementary Table S1), with a stronger effect for the complete resolution group.

HLA-DPB1 rs9277535 and HBV Infection and Clearance

Similar analyses for *HLA - DPB1* rs9277535 indicated a weaker protective association (OR, .81; $P = .036$; additive model) with chronic HBV infection and with failure of HBV clearance (OR, 1.29; $P = .004$; additive model) (Supplementary Table S2). This SNP had stronger association in the Japanese group [9], raising the possibility of different degrees of linkage disequilibrium (LD) between Chinese and Japanese populations. Indeed, comparison of LD structure between 2 populations with use of 55 HapMap genotyped SNPs in the *HLA - DPA1*/*DPB1* region indicated that the LD level in Chinese tends to be lower than that in Japanese between *HLA-DP1* and *HLA - DPB1* (Supplementary Fig. S1A and S1B); the level of LD between rs3077 in *HLA-DP1* and rs9277535 in *HLA - DPB1* is weaker in Chinese ($D' = .75$; $r^2 = .40$) than in Japanese ($D' = .87$; $r^2 = .67$). This finding suggests that rs3077 is more likely to be either functional or tracking causal variation, because its effects are consistent in both the Japanese and Chinese Han populations, unlike rs9277535.

HLA-DPA1 rs3077 and Development of Cirrhosis and HCC

Because the impact on HBV infection and clearance is so pronounced for *HLA - DPA1* rs3077, we hypothesized that, in the context of chronic HBV infection, the *HLA - DPA1* locus might influence progression to more severe outcomes of chronic HBV infection by controlling HBV replication and/or modulating immune inflammatory response. However, a comparison of 370 patients with cirrhosis with 590 patients with chronic HBV infection without cirrhosis revealed no significant associations (Table 2). The results were similar when patients with chronic HBV infection were compared with patients with the more severe phenotype of decompensated cirrhosis (data not shown). With these sample sizes, we had 86% and 83% power to detect an association with an OR of 1.5 with cirrhosis and HCC, respectively. When comparing 265 patients with HCC with 961 patients with chronic HBV infection and cirrhosis , no association of rs3077 was observed (Table 2). Consistent with *HLA - DPA1* rs3770, no associations with the more severe outcomes of cirrhosis or HCC were detected for *HLA - DPB1* rs9277535 (Supplementary Table S2).

Potential Regulatory Function of *HLA-DPA1* rs3077

We explored the potential function of rs3077 through several in-silicon bioinformatic tools. The SNP rs3077 T was found to be associated with higher expression levels of *HLA - DPA1* in peripheral blood mononuclear cells of European descent (Supplementary Figure S2A) by a search in SNPExpress, a database of the transcript and exon levels of gene expression as measured by genome-wide gene expression and genotypes [13]. It remains to be demonstrated whether this association was also present in Han Chinese samples because of the high level of diversity at the *HLA* region. There are several mechanisms by which *HLA - DPA1* rs3077 may regulate expression levels: a microRNA binding site is disrupted, which may affect microRNA binding, leading to repressed translation or destabilized mRNA (Supplementary Figure S2B), or could alter transcription factor binding (Supplementary Figure S2C).

DISCUSSION

Class II HLA molecules, such as HLA-DP, present antigen to $CD4^+$ T helper cells. T cell helper response is crucial in HBV clearance, as evidenced by the vigorous humoral immune response observed in patients achieving spontaneous clearance [14]. In our study, we replicated the results observed in the Kamatani et al [9] genome-wide association study, showing that individuals carrying the rs3077 C allele were susceptible to HBV infection, and we further showed, for the first time to our knowlegde, that rs3077 C allele is also strongly associated with failure to clear HBV infection. Furthermore, comparison of the strength and effect size of rs3077 on HBV infection and clearance in our study indicate that the rs3077T protective effect is mainly attributable to more efficient viral clearance, rather than decreased susceptibility to infection, an aspect that was not determined in the previous study [9]. Our study, together with the study by Kamatani et al [9], indicates a major role of HLA - DPA1 in modulation of HBV acquisition and resolution of HBV infection.

The impact of rs3077C on the burden of HBV infection in Asian populations is substantial, considering its high allele frequency. The carriage of rs3077C may affect epidemic HBV infection, because the increasing susceptibility of individuals carrying the allele increases the number of transmitters and the likelihood of a recipient developing a chronic infection. Other environmental and genetic factors that contribute to HBV infection remain to be identified.

SNP rs3077, located in the 3′ untranslated region of *HLA - DPA1*, may be tracking *HLA - DPA1* alleles that bind relevant HBV epitopes or tracking a variant in a regulatory element

that affects *HLA - DPA1* stability or expression. However, there is evidence that the rs3077 C allele is associated with lower *HLA - DPA1* expression, suggesting that rs3077 risk allele or its linked true regulatory SNP plays its role by down regulating *HLA - DPA1* expression. Lower levels of HLA - DPA1 on target cell surfaces might be less effective in presenting viral antigen to $CD4^+$ T helper cells, leading to an impaired immune response to viral invasion or to the resolution of HBV infection. The reason why this SNP has no obvious impact on disease progression to cirrhosis or HCC remains to be explored. Further studies on HBV disease progression, treatment response, and vaccine response are warranted. Vaccine response to HBsAg is controlled in part by the *HLA-DR* region [15], but the role of *HLA - DPA1* in vaccine response has not been investigated. Elucidation of the mechanism of HLA-DP interaction with HBV will be a critical step in our understanding of immune regulation of HBV infection, clearance, and vaccine response and may lead to targeted epidemic control strategies.

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Supplementary Data

Supplementary data are available at http://jid.oxfordjournals.org online.

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