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Association of common promoter polymorphisms of *MCP1* with hepatitis B virus clearance

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Abbreviations: CH, chronic hepatitis; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LC, liver cirrhosis; SNP, single nucleotide polymorphism

Abstract

Hepatocellular carcinoma (HCC) is one of the most common malignant cancers closely associated with chronic infection by the hepatitis B virus (HBV) or the hepatitis C virus (HCV) throughout the world. In this study, the genetic associations of 20 known polymorphisms in eight candidate genes, including angiotensinogen (*AGT*), cadherin 1 (*CDH1*), cyclooxygenase 2 (*COX2*), monocyte chemotactic protein-1 (*MCP1*), multidrug resistance 1 (*MDR1*), chemokine ligand 5 (*RANTES*), thrombospondin 2 (*THBS2*), and thrombospondin 4 (*THBS4*), were analyzed in a large chronic hepatitis B cohort (*n* = 1,095) recruited from the Korean population. In addition, three polymorphisms in chemokine receptor 4 (*CXCR4*) and vimentin (*VIM*) identified in this study were also genotyped. Using logistic regression analysis controlling possible confounding factors, one common (freq.=0.367) promoter polymorphism of *MCP1* (*MCP1-2518G>A*) among analyzed polymorphisms was significantly associated with clearance of HBV infection. The frequency of homozygotes for the *MCP1-2518A* allele (*MCP1-2518A/A*) among chronic hepatitis B virus (HBV) carrier patients was significantly higher than that among spontaneously recovered (SR) subjects (17.7% vs. 10.4%)(OR=1.78, *P* = 0.004). Our findings imply a plausible explanation for the contribution of host genetic determinants to the variable outcome of HBV infection, which might provide valuable information for future genetic study in this area.

Keywords: angiotensinogen; cadherins; cyclooxygenase 2; carcinoma, hepatocellular; chemokine CCL2; hepatitis B virus; P-glycoprotein; polymorphism, single nucleotide; RANTES; thrombospondins

Introduction

Chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) has a major role in the development of hepatocellular carcinoma (HCC) (Blumberg, 1984). Although the virologic features of the two viruses are entirely different, both viruses infect the human liver and initiate a series of processes leading to chronic hepatitis, cirrhosis, and HCC (Watson, 1999). Some epidemiological findings suggest different modes of disease progression and HCC promotion between HBV and HCV infection (Shiratori *et al.,* 1995; Ikeda *et al.,* 1998; Marotta *et al.,* 2004). The mechanisms underlying such differences are unknown.

The clinical course of HBV infection varies from spontaneous recovery after acute hepatitis to a chronic persistent infection that may progress to cirrhosis or hepatocellular carcinoma. The mechanisms underlying resolution of acute HBV infection or its progression to chronicity remain undetermined. Age at infection has the most significant impact on the clinical outcome because chronic infection occurs in approximately 90% of infants infected at birth, in 25 to 50% of children infected between the ages of 1 and 5 years, and in less than 5% of those infected during adult life (Stevens *et al.,* 1975; Coursaget *et al.,* 1987; Tassopoulos *et al.,* 1987). It is well known that the major mode of infection in HBV endemic areas, including Korea, is perinatal transmission (Stevens *et al.,* 1975; Lok *et al.,* 1987). The mechanisms underlying resolution of acute HBV infection or its progression to chronicity at each age group remain undetermined. When determining the chronicity of HBV infection within a group of patients who are presumed to have been infected at the same age, *i.e.* perinatally in Korea, it is apparent that the

outcome of the infection does not appear to be determined by variations in virulence of the viral strains (Thursz *et al.,* 1995; Cacciola *et al.,* 2002), but that host factors are likely to influence disease outcome (Lin *et al.,* 1989; Chisari and Ferrari 1995). Thus, it is conceivable that genetic differences play an additional role.

In recent years, a number of studies have shown that various genes could be involved in chronic liver diseases, liver regeneration, autoimmune hepatitis, primary biliary cirrhosis, and alcoholic liver disease, including monocyte chemotactic protein-1 (*MCP1*) (Marra *et al.,* 1998), angiotensinogen (*AGT*) (Ishii *et al.,* 2003; Medina *et al.,* 2003; 2004; Moon *et al.,* 2004), cadherin 1 (*CDH1*) (Tian *et al.,* 1999; Wei *et al.,* 2002), cyclooxygenase 2 (*COX2*) (Shiota *et al.,* 1999), multidrug resistance 1 (*MDR1*) (Bao *et al.,* 2000; Ros *et al.,* 2003), chemokine ligand 5 (*RANTES*) (Apolinario Fernandez de Sousa and Garcia Monzon, 2003), thrombospondin 2 (*THBS2*), thrombospondin 4 (*THBS4*) (Ishii *et al.,* 2003; Medina *et al.,* 2003; 2004; Moon *et al.,* 2004), chemokine receptor 4 (*CXCR4*) (Terada *et al.,* 2003; Wald *et al.,* 2004), and vimentin (*VIM*) (O'Brien *et al.,* 1989).

MCP1 is structurally related to the CXC subfamily of cytokines (Copple *et al.,* 2003). Secretion of MCP1 may contribute to the formation and maintenance of the inflammatory infiltrate observed during chronic liver disease (Marra *et al.,* 1998). Alcoholic hepatitis and alcoholic cirrhosis are associated with distinct patterns of chemokine expression that are likely to be important factors in determining whether a patient develops acute parenchymal inflammation and alcoholic hepatitis, or chronic septal inflammation and alcoholic cirrhosis (Afford *et al.,* 1998).

Angiotensinogen (AGT) is expressed in the liver and is cleaved by the enzyme renin in response to lowered blood pressure. AGT is involved in maintaining blood pressure and in the pathogenesis of essential hypertension and preeclampsia (Morgan *et al.,* 1996). A statistically significant relationship was seen between inheritance of *AGT*-producing genotypes and the development of progressive hepatic fibrosis (Powell *et al.,* 2000).

THBS2 and THBS4 belong to the thrombospondin family of disulfide-linked homotrimeric glycoproteins that mediate cell-to-cell and cell-to-matrix interactions. These proteins have been shown to function as potent inhibitors of tumor growth and angiogenesis (Kishi *et al.,* 2003; Wessel *et al.,* 2004). Previous studies have shown the expression and function of many pro- and antiangiogenic molecules in the setting of nontumoral chronic liver diseases and disease progression (Ishii *et al.,* 2003; Medina *et al.,* 2003; 2004; Moon *et al.,* 2004).

Hepatic RANTES was found to be increased in patients with alcoholic hepatitis (Hirano *et al.,* 2003). Wald *et al.* (2004) also showed an important role for the CXCR4 in recruitment and retention of immune cells in the liver during chronic HCV and HBV infection. Similarly, MDR1 and VIM were up-regulated in hepatocytes with severe liver disease (Ros *et al.,* 2003) and in hepatoblastomas where mesenchymal tissue was present in the tumor (O'Brien *et al.,* 1989).

CDH1 is a calcium-dependent cell-cell adhesion glycoprotein composed of five extracellular cadherin repeats, a transmembrane region and a highly conserved cytoplasmic tail. Mutations in this gene are correlated with gastric, breast, colorectal, thyroid, and ovarian cancers. Loss of function is thought to contribute to progression in cancer by increasing proliferation, invasion, and/or metastasis (Juhasz *et al.,* 2003; Keller and Nigam 2003; Kowalski *et al.,* 2003).

As a candidate gene association study, we investigated the genetic association of 23 polymorphisms in 10 candidate genes with different outcomes of HBV infection, including clearance of HBV and HCC occurrence.

Materials and Methods

Study population and outcomes

A total of 1,095 Korean subjects having either

Table 1. Clinical profiles of study subjects.

Clinical profiles	SR	CC	
		CH or LC	HCC
No. of subjects	429	339	327
Age (mean (range))	54.9 (28-79)	49.9 (22-85)	58.3 (25-79)
Sex (male/female)	240/189	274/65	277/50
HBeAg (positive rate, %)	0	33.5	19.6
HBeAb (positive rate, %)	38.2	47.3	63.8
HBsAg (positive rate, %)	0	100.0	100.0
HBsAb (positive rate, %)	100.0	0	0
Urine albumin (positive rate, %)	0	8.5	15.0
Urine blood (positive rate, %)	28.7	14.7	24.8

SR, spontaneous recovery; CC, chromic carrier; CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma

present or past evidence of HBV infection were prospectively enrolled from the outpatient clinic of the liver unit or from the Center for Health Promotion of Seoul National University Hospital between January 2001 and August 2003. Subjects were placed in two different groups according to serologic markers: the chronic carrier (CC) group, or the spontaneous recovery (SR) group. The CC and SR cohorts consisted of 666 and 429 subjects, respectively (Table 1). The diagnoses of the CC and SR subjects were established by repeated seropositivity for the hepatitis B surface antigen (HBsAg) (Enzygnost® HBsAg 5.0; Dade Behring, Marburg, Germany) over a 6-month period, and for both anti-HBs (Enzygnost® Anti-HBs II; Dade Behring, Marburg, Germany) and anti-HBc (AB-Corek; Dia-Sorin s.r.l., Saluggia, Italy) of the IgG type without HBsAg, respectively. Asymptomatic HBV carriers were also included in CC group. These patients usually have inactive liver disease on liver biopsy. However, it has been known that in these patients, HBV continues to replicate, albeit very low levels, some patients have residual liver disease, and HCC develop frequently. The CC group was further divided into 2 subgroups, *i.e.*, those without (the CH/LC group; $n = 339$) and those with HCC (the HCC group; $n = 327$), according to the absence or presence of HCC, respectively. HCC was diagnosed as described previously (Bruix *et al.,* 2001). We excluded subjects who were positive for anti-HBs but not for anti-HBc, and those positive for anti-HCV or anti-HIV (GENEDIA®; Greencross Life Science Corp., Yongin-shi, Korea, HCV®3.2; Dong-A Pharmaceutical Co., Seoul, Korea). The patients who had any other types of liver disease such as autoimmune hepatitis, toxic hepatitis, primary biliary cirrhosis, or Budd-Chiari syndrome were also excluded. No patients had a previous history of immunosuppression or anti-viral treatment. Informed consent was obtained from each patient, and the Institutional Review Board of Human Research at Seoul National University Hospital approved the study protocol. The clinical parameters are summarized in Table 1.

Sequence analysis of the human *CXCR4* and *VIM*

We have sequenced exons and their flanking regions, including the promoter region (1.5 kb), to discover variants in 24 Korean unregulated individual DNA samples using the ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, CA). Primer sets for the amplification and sequencing analysis of *CXCR4* and *VIM* were designed based on GenBank sequences (Ref. Genome seq.; NT_005058 released on 19. Aug. 2004 and NT_077569 released on 20. Aug. 2004, respectively). Information regarding primers is available on our website (http://www.snp-genetics.com/reference/Supplementary information to HBV.doc). Sequence variants were verified by chromatograms.

Genotyping with fluorescence polarization detection

Twenty polymorphisms of eight candidate genes that might be implicated in clearance of HBV infection and HCC occurrence were genotyped in this study, including *-532C>T, -217G>A, -6A>G,* and *+3889C>T* in *AGT -472insdelA, -285C>A, +76003A>C, +84762C>G,* and *+86123C>T* in *CDH1* (Humar *et al.,* 2002; Nakamura *et al.,* 2002; Wang *et al.,* 2003); *-162C>G, +3552G>A,* and *+5789T>C* in *COX2* (Fritsche *et al.,* 2001); *-2518G>A* and *-2076A>T* in MCP1 (Aguilar *et al.,* 2001; Szalai *et al.,* 2001; Kim *et al.,* 2002); *+69219G>T* and *+91191T>C* in *MDR1* (Jamroziak *et al.*, 2004; Kajinami *et al.*, 2004); *-403G>A* and *-28C>G* in *RANTES* (Liu *et al.,* 1999); *+36412T>G* in *THBS2*; and *+30275G>C* in *THBS4* (Topol *et al.*, 2001; Boekholdt *et al.*, 2002). In addition, three polymorphisms in chemokine receptor 4 (*CXCR4*) and vimentin (*VIM*), identified in this study, were also genotyped (Table 2).

For genotyping of 23 polymorphic sites, amplifying primers and probes were designed for TaqMan® (An *et al.,* 2002). Primer Express (Applied Biosystems) was used to design both the PCR primers and the MGB TaqMan probes. One allelic probe was labeled with the FAM dye and the other with the fluorescent VIC dye. Typically, PCR was run in the TaqMan Universal Master mix without UNG (Applied Biosystems) at primer concentration of 900 nM and TaqMan MGB-probe concentration of 200 nM. The reaction was performed in a 384-well format in a total reaction volume of 5 μl using 20 ng of genomic DNA. The plate was then placed in a thermal cycler (PE 9700, Applied Biosystems) and heated for 2 min at 50°C and for 10 min at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The TaqMan assay plate was then transferred to a Prism 7900HT instrument (Applied Biosystems) where the fluorescence intensity of each well was read. Fluorescence data files from each plate were analyzed by automated software (SDS 2.1). Detailed information concerning the primers can be obtained at the website mentioned above.

Statistics

We examined Lewontin's D' (|D'|) and LD coefficient r^2 between all pairs of biallelic loci (Hedrick, 1987). Haplotypes of each individual were inferred using the algorithm developed by Stephens *et al.* (2001),

Table 2. Analysis for HBV clearance and HCC occurrence on CH or LC with SNPs and haplotypes in candidate genes in Korean subjects.

Gene	Allele	Allele	HBV clearance			HCC occurrence		
			Allele distribution [n (%)]		Permutation *P*-value	Allele distribution [n (%)]		Permutation *P*-value
			CC	SR		HCC	No HCC	
*CXCR4***	*+2547C>T*	C	1,166 (89.6)	767 (89.4)	0.94	566 (89.8)	587 (89.2)	0.71
		T	136 (10.5)	91 (10.6)		64 (10.2)	71 (10.8)	
*VIM***	*-510A>G*	A	839 (71.8)	603 (72.0)	0.96	425 (73.0)	403 (70.5)	0.36
		G	329 (28.2)	235 (28.0)		157 (27.0)	169 (29.6)	
	+6750G>C	G	862 (70.9)	605 (70.8)	1.00	432 (72.2)	419 (69.4)	0.28
		C	354 (29.1)	249 (29.2)		166 (27.8)	185 (30.6)	
*AGT**	*-532C>T*	C	999 (83.3)	694 (82.2)	0.56	498 (83.0)	488 (83.3)	0.94
		T	201 (16.8)	150 (17.8)		102 (17.0)	98 (16.7)	
	-217G>A	G	950 (83.6)	701 (83.1)	0.76	485 (83.6)	452 (83.4)	0.93
		A	186 (16.4)	143 (16.9)		95 (16.4)	90 (16.6)	
	-6A>G	A	1,010 (80.2)	687 (80.1)	0.95	467 (80.0)	531 (80.2)	0.94
		G	250 (19.8)	171 (19.9)		117 (20.0)	131 (19.8)	
	+3889C>T	C	1,135 (89.1)	765 (89.0)	0.94	550 (90.2)	571 (87.9)	0.20
		T	139 (10.9)	95 (11.1)		60 (9.8)	79 (12.2)	
	ht3	-/-	999 (83.7)	706 (82.5)	0.75	498 (83.6)	488 (83.6)	0.35
		ht3/ht3	195 (16.3)	150 (17.5)		98 (16.4)	96 (16.4)	
*CDH1***	*-472insdelA*	DEL	912 (74.4)	647 (75.2)	0.68	447 (76.5)	454 (72.3)	0.10
		INS	314 (25.6)	213 (24.8)		137 (23.5)	174 (27.7)	
	-285C>A	C	1,058 (82.5)	694 (80.7)	0.30	498 (81.6)	549 (83.4)	0.41
		A	224 (17.5)	166 (19.3)		112 (18.4)	109 (16.6)	
	+76003A>C	A	1,268 (99.8)	857 (99.9)	1.00	605 (99.8)	649 (99.9)	1.00
		C	2 (0.2)	1 (0.1)		1 (0.2)	1 (0.2)	
	+84762C>G	C	1,307 (99.2)	849 (99.0)	0.65	632 (98.8)	661 (99.6)	0.14
		G	11 (0.8)	9 (1.1)		8 (1.3)	3 (0.5)	
	+86123C>T	C	783 (61.2)	505 (58.7)	0.27	371 (60.4)	402 (61.7)	0.69
		T	497 (38.8)	355 (41.3)		243 (39.6)	250 (38.3)	
	ht3	-/-	980 (78.0)	690 (80.2)	0.24	470 (77.8)	500 (78.4)	0.84
		ht3/ht3	276 (22.0)	170 (19.8)		134 (22.2)	138 (21.6)	
*COX2**	*-162C>G*	C	1,251 (99.3)	846 (98.8)	0.35	608 (99.0)	629 (99.5)	0.33
		G	9 (0.7)	10 (1.2)		6 (1.0)	3 (0.5)	
	+3552G>A	G	1,297 (99.3)	847 (99.0)	0.47	612 (99.0)	671 (99.6)	0.32
		A	9 (0.7)	9 (1.1)		6 (1.0)	3 (0.5)	
	+5789T>C	T	1,283 (99.9)	858 (100.0)	1.00	613 (99.8)	656 (100.0)	0.48
		C	1 (0.1)	0 (0.0)		1 (0.2)	0 (0.0)	
*MCP1**	*-2518G>A*	**G**	**742 (61.2)**	**566 (66.6)**	**0.01**	367 (61.4)	366 (61.0)	0.91
		A	**470 (38.8)**	**284 (33.4)**		231 (38.6)	234 (39.0)	
	-2076A>T	A	1,214 (93.5)	812 (94.6)	0.31	579 (93.7)	622 (93.4)	0.91
		T	84 (6.5)	46 (5.4)		39 (6.3)	44 (6.6)	
	ht2	-/-	818 (67.9)	611 (71.9)	0.06	403 (68.3)	405 (67.5)	0.81
		ht2/ht2	386 (32.1)	239 (28.1)		187 (31.7)	195 (32.5)	
*MDR1***	*+69219 G>T*	G	586 (54.2)	448 (53.9)	0.92	268 (53.0)	311 (55.3)	0.45
		T	496 (45.8)	384 (46.2)		238 (47.0)	251 (44.7)	
	+91191T>C	T	840 (63.8)	547 (63.9)	1.00	392 (61.8)	440 (65.9)	0.13
		C	476 (36.2)	309 (36.1)		242 (38.2)	228 (34.1)	
	ht3	-/-	963 (89.0)	733 (88.3)	0.66	454 (89.7)	497 (88.4)	0.55
		ht3/ht3	119 (11.0)	97 (11.7)		52 (10.3)	65 (11.6)	
*RANTES**	*-403G>A*	G	755 (58.5)	508 (59.5)	0.69	361 (58.6)	387 (58.6)	1.00
		A	535 (41.5)	346 (40.5)		255 (41.4)	273 (41.4)	
	-28C>G	C	668 (79.9)	666 (83.3)	0.09	307 (79.1)	353 (80.6)	0.60
		G	168 (20.1)	134 (16.8)		81 (20.9)	85 (19.4)	
	ht2	-/-	651 (78.1)	609 (76.5)	0.47	296 (76.7)	348 (79.5)	0.34
		ht2/ht2	183 (21.9)	187 (23.5)		90 (23.3)	90 (20.6)	
*THBS2***	*+36412T>G*	T	1,155 (89.5)	769 (90.1)	0.71	548 (90.4)	594 (88.7)	0.31
		G	135 (10.5)	85 (10.0)		58 (9.6)	76 (11.3)	
*THBS4***	*+30275G>C*	G	1,232 (93.8)	819 (95.5)	0.10	589 (93.8)	630 (93.8)	1.00
		C	82 (6.2)	39 (4.6)		39 (6.2)	42 (6.3)	

*Transcriptional start site is denoted by +1. **Translational start site is denoted by +1. Permutation *P*-values were calculated by SAS. All patients included in study were HBsAg-positive (chronic hepatitis).

Table 3. Logistic analysis of clearance of HBV with MCP1 gene polymorphisms in Korean subjects.

Locus	Genotype	Genotype distribution [n(%)]		Referent		Co-dominant		Dominant		Recessive	
		CC	SR	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
-2518G>A	*GG*	**243 (40.1)**	**185 (43.5)**	1							
	AG	**256 (42.2)**	**196 (46.1)**	0.94 (0.71-1.25)	0.67	1.20 (0.99-1.44)	0.06	1.08 (0.83-1.41)	0.56	**1.78 (1.20-2.63)**	**0.004**
	AA	**107 (17.7)**	**44 (10.4)**	**1.32 (1.07-1.62)**	**0.009**						
-2076A>T	*AA*	568 (87.5)	385 (89.7)	1							
	AT	78 (12.0)	42 (9.8)	1.40 (0.92-2.14)	0.11	1.38 (0.94-2.03)	0.10	1.41 (0.94-2.13)	0.10	1.50 (0.23-9.79)	0.67
	TT	3 (0.5)	2 (0.5)	1.25 (0.49-3.18)	0.65						
ht2	*-/-*	**293 (48.7)**	**213 (50.1)**	1							
	-/ht2	**232 (38.5)**	**185 (43.5)**	0.83 (0.63-1.10)	0.19	1.11 (0.91-1.35)	0.33	0.96 (0.74-1.24)	0.74	**1.92 (1.19-3.08)**	**0.007**
	ht2/ht2	**77 (12.8)**	**27 (6.4)**	**1.34 (1.05-1.71)**	**0.02**						

Logistic regression models were used for calculating odds ratios (95% confidence interval) and corresponding *P*-values for each SNP site and haplotype using SAS. *P*-values of co-dominant, dominant, and recessive models are also given. Age (continuous value) and sex (male = 0, female = 1) were adjusted by inclusion in logistic analysis as covariates.

which (PHASE) uses a Bayesian approach incorporating a priori expectations of haplotypic structure from population genetic and coalescent theory. Genetic effects of inferred haplotypes were analyzed in the same way as SNPs. The permutation test was also performed to test deviation of allelic frequencies of SNPs and haplotypes by using SAS (proc multtest). Distribution was estimated by evaluating the statistics for a random sampling of 10,000 iterated permutations at fixing the total numbers of both the cases and controls. *P*-value is estimated by the proportion of permutations for which the permutated data test statistic ($P_{permuted}$) is greater than the initially observed test statistic ($P_{observed}$), so permutation $P = P$ ($P_{observed} > P_{permuted}$) (Table 2).

Logistic regression models were used for calculating odds ratios (95% confidential interval) and corresponding *P*-values controlling for age (continuous value) and sex (male = 0, female = 1) as covariates. In our analysis of HCC occurrence, LC (LC = 1, no LC = 0) and HBeAg (negative = 0, blank = 1, positive = 2) were also used as covariates (Table 3).

Results

The first critical step in conducting candidate gene studies is the choice of suitable candidate genes that may plausibly play a relevant role in the process or disease under investigation. The candidate gene approach for discovering genetic markers uses experimentally derived a priori knowledge about diseases, and we selected ten candidate genes and their polymorphisms, previously reported the relationship to the various liver diseases, for association study with HBV infection and HCC occurrence.

To discover polymorphisms in *CXCR4* and *VIM*, we performed direct DNA sequencing in 24 unrelated Korean individuals within exons and their boundaries, including the 1.5 kb upstream region of two genes. We identified one sequence variant (*+2547C>T*) in exon2 of *CXCR4* and five variants (two in promoter [*-510A>G* and *-312G>C*], one in 5' UTR [*-22G>C*], and two in introns [*+6750G>C* and *+7379C>T*]) in *VIM,* respectively (http://www.snp-genetics.com/reference/Supplementary information to HBV.doc). *CXCR4+2547C>T, VIM -510A>G,* and *VIM +6750G>C* were selected for larger-scale genotyping (n = 1,095), considering their allele frequencies, haplotype-tagging status, and LDs among polymorphisms. Allele frequencies of all of screened polymorphisms were estimated (http://www.snp-genetics.com/reference/Supplementary information to HBV.doc).

Through pair-wise linkage analysis in a Korean chronic hepatitis B cohort, we have found that three sets of polymorphisms in *COX2* and two of *MCP1*

were in complete LD (|D'| = 1 and $r^2 \neq 1$), and that four sets of polymorphisms in *AGT* were in almost complete LD. Another set of polymorphisms in *CDH1*, *RANTES*, *MDR1,* and *VIM* showed strong LDs within genes (http://www.snp-genetics.com/reference/Supplementary information to HBV.doc). Among the haplotypes, only *ht2* in *MCP1*, *ht1* in *AGT*, *ht1* in *CDH1*, *ht3* in *MDR1,* and *ht2* in *RANTES* were used for further analysis because other haplotypes were almost (or completely) tagged by a single SNP or the frequencies were too low.

Genotype distributions were analyzed among SR, CC, and HCC subjects using logistic regression models. One common promoter polymorphism in *MCP1* (*MCP1-2518G>A)* was found to be significantly associated with clearance of HBV infection, whereas no significant associations were observed between other candidate gene polymorphisms and the clearance of HBV infection (Table 2). The frequency of *MCP1-2518A* allele (*MCP1-2518A/A*) among the chronic hepatitis B virus (HBV) carrier patients was significantly higher than for the spontaneously recovered (SR) subjects (38.8% vs. 33.4%) (permutation $P = 0.01$). Similarly, the effect of the homozygote for the "*A*" allele of *-2518G/A* also was apparent in the referent model (OR = 1.32, $P = 0.009$, Table 3). Consistent associations revealed in referent- and recessive-analyzing models clearly demonstrated the recessive mode of effect of the "*A*" allele of *-2518G/A*. In further haplotype association analysis, *ht2* [*A-A*] showed similar association with clearance of HBV. However, considering that *ht2* is mostly (> 93%) tagged by *2518G>A,* this genetic effect might be coming from *-2518G>A.* In the analysis of HCC occurrence in Korean population, all candidate gene polymorphisms were not associated (Table 2).

Discussion

MCP1, a member of the small inducible gene (SIG) family, plays a role in the recruitment of monocytes to sites of injury and infection. It has been reported that *MCP* polymorphisms in the regulatory region affect the level of MCP1 expression in response to an inflammatory stimulus. Interestingly, the associated polymorphism *(MCP1-2518G>A)*, which showed significant association with clearance of HBV, has been reported to alter MCP1 expression, whereas no effect by the other promoter variant *(MCP1-2076A>T)* has been reported (Rovin *et al.,* 1999). IL1B-treated peripheral blood mononuclear cells from individuals heterozygous or homozygous for the G allele at *MCP1-2518G>A* produce more MCP1 than cells from individuals homozygous for the A allele (Rovin *et al.,* 1999; Cho *et al.,* 2004). Altered level of MCP1 expression due to genotype allele could contribute to the formation and maintenance of the inflammatory infiltrate during chronic liver disease and severe hepatic inflammation and fibrosis (Marra *et al.,* 1998; Muhlbauer *et al.,* 2003). Involvement of *MCP1* polymorphisms in the regulatory region has been reported in various diseases including asthma (Szalai *et al.,* 2001), systemic lupus erythematosus (Aguilar *et al.,* 2001; Kim *et al.,* 2002), HCV progression (Muhlbauer *et al.,* 2003; Tagami *et al.,* 2003), and HBV infection in this study. Different MCP-1 expressions by alternative alleles of *-2518G>A* (Rovin *et al.,* 1999; Muhlbauer *et al.,* 2003) consistently suggest functional and/or causal effects of this important variant.

The functional effects of *MCP1-2518G>A* on the progression and severity of various diseases were mainly accompanied by the elevated levels of MCP1 due to *G* allele of *MCP1-2518G>A*, whereas A allele of *MCP1-2518G>A*, causing lower level of MCP1, contributed to the chronicity of HBV in this study. Although the mechanisms underlying clearance of HBV infection by lower MCP1 expression of the "A" allele of *MCP1-2518G>A* are not completely understood, several clues might suggest possible explanations. For instance, MCP-1 might contribute to the formation and maintenance of the inflammatory infiltrate observed during chronic liver disease (Marra *et al.,* 1998). In an animal study, MCP1-deficient mice were unable to mount type 2 helper cell (TH2) responses (Gu *et al.,* 2000). Consequently, these mice did not accomplish the immunoglobulin subclass switch that is characteristic of TH2 responses and were resistant to Leishmania major. MCP1 influences both innate immunity, through effects on monocytes, and adaptive immunity, through control of T helper cell polarization (Gu *et al.,* 2000).

The effects of *MCP1* polymorphisms on the resolution of HBV infection were not dramatic in the present study. However, when considering 1) that there were evidences for functional differences mediated by *MCP1-2518G>A* (Rovin *et al.,* 1999; Cho *et al.,* 2004), consequently, affecting disease susceptibility of various diseases in previous studies (Aguilar *et al.;* 2001; Szalai *et al.,* 2001; Kim *et al.,* 2002), (Rovin *et al.,* 1999; Muhlbauer *et al.,* 2003; Tagami *et al.,* 2003), and 2) that altered level of MCP1 expression have been reported to be involved in the inflammatory infiltrate during chronic liver disease and severe hepatic inflammation and fibrosis (Marra *et al.,* 1998; Muhlbauer *et al.,* 2003), the significance of associations with the clearance of HBV infection might be noteworthy. Further biological and/or functional evidence would be needed to

confirm the suggestive associations of *MCP1* polymorphisms with HBV infection.

In summary, we examined the genetic association of 23 polymorphisms in ten candidate genes. Among 23 polymorphisms, one common promoter variant in *MCP1* (*-2518G>A)*, which is associated with lower MCP1 expression, was significantly associated with the resolution of HBV infection in a Korean HBV study. Haplotype analysis revealed that *MCP1-ht2* [*A-A*] was also significantly associated with HBV clearance, which possibly tracks the genetic effect of the promoter variant. Our findings imply that variations in the genes governing the level of constitutive and inducible MCP1 are an important factor that might explain the variable outcome of HBV infection and offer an approach to elucidating the molecular mechanisms of HBV clearance. This finding might also provide a new perspective on antiviral approaches for the treatment of patients with chronic hepatitis B.

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