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Putative Association of *ITGB1* Haplotype with the Clearance of HBV Infection

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Abstract

Integrins are transmembrane receptor proteins that mediate cell-cell adhesion and cell-extracellular matrix (ECM) adhesion. The deregulation of cell-ECM adhesion and the abnormal expression of beta1 (β1) integrins (*ITGB1*s) are involved in tumor development and metastasis. In the liver, the expression of integrins and ECM proteins can be a cause of hepatocellular carcinoma (HCC) development. We performed direct DNA sequencing of 24 individuals, and identified 23 sequence variants of *ITGB1* polymorphisms. Among these 23 variants, 7 common variants were selected based on frequencies and linkage disequilibrium, and then genotyped in a larger-scale group of subjects (n=1,103). The genetic associations of *ITGB1* polymorphisms with the clearance of HBV and HCC outcome of HBV patients were analyzed using logistic regression models and Cox relative hazard models. Although there was no significant association observed between the polymorphisms and the HCC outcome of HBV patients, the second most common haplotype (*ITGB1 haplotype-2 [C-C-C-C-T-C-T]*) was putatively associated with HBV clearance (OR=0.75, p=0.008 and P^{corr}=0.05). The minor allele frequency (MAF) of *ITGB1 haplotype*-2 of the spontaneously recovered (SR) group was significantly higher than that of the chronic carrier group (CC) (freq. = 0.248 vs. 0.199). The information derived from this study could be valuable for understanding the genetic factors involved in the clearance of HBV.

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Introduction

The hepatitis B virus (HBV) is a crucial factor in bringing about acute and chronic liver diseases (Lin *et al*., 2005). Approximately 350 million people are chronically infected with HBV all over the world. In fact, more than 1 million HBV carriers die annually because of HBV-related diseases such as liver cancer (Chang *et al*., 2005). Each year, nearly 50 million cases are newly diagnosed, and out of that, 90% of the affected individuals were infants while the remaining 5～10% were adults (Merican *et al*., 2000). Continuous HBV infection can cause liver cirrhosis (LC) and hepatocellular carcinoma (HCC) (Lin *et al*., 2005). HCC is the most widespread and severe form of malignancies that are diagnosed in adults. Patients infected with the hepatitis B or C virus are more likely to develop HCC, which in turn is accompanied by liver cirrhosis. The progression of HCC results in a stepwise series of events. Each step in the development of HCC appears to be linked to separate genetic and epigenetic aberrations. These changes are associated with alterations in the expression or formation of an oncogene or a tumor suppressor gene (Patil *et al*., 2009). In addition, several previous studies have also reported associations between genetic polymorphisms and the risk of HCC and/or HBV clearance, e.g., histone deacetylase-10 (*HDAC10*) and secreted phosphoprotein-1 (*SPP1*) polymorphisms, and interleukin-10 (*IL10*) haplotypes were also shown to be associated with HBV clearance and/or HCC development (Lin *et al*., 2005; Park *et al*., 2007; Shin *et al*., 2003; Shin *et al*., 2007).

Members of the integrin family, the beta1 (β1) integrins (ITGB1; MIM# 135630) are heterodimeric structures consisting of a common β1 subunit that is non-covalently associated with one of nine different α subunits. These molecules are widely distributed in various cells and they mediate cell-cell and cell-ECM interactions that are related to many biological functions in the development of cell or tissues, hemostasis and immune response (Garrido *et al*., 2001). It has been shown that the deregulation of cell adhesion to the ECM and the abnormal expression of ITGB1s, particularly the α5 β1 down-regulation, are closely associated with tumor development and metastasis. Especially in the liver, the

expression of integrins and ECM proteins has been linked to HBV infection and HCC development (Lara-Pezzi *et al*., 2001; Lee *et al*., 2009).

Based on these observations in the area of tumor and cancer development, we hypothesized that the polymorphisms in the *ITGB1* gene could influence the clearance of HBV and HCC progression among HBV-infected patients. We performed extensive screening of *ITGB1* by direct sequencing to detect polymorphisms, and we examined their genetic associations with HBV clearance and HCC progression. Here, we report 23 genetic polymorphisms identified in *ITGB1* and their genetic associations with HBV clearance and HCC progression in a Korean population (n=1,103).

Methods

Subjects and outcomes

A total of 1,103 Korean subjects having either present or past evidence of HBV infection were prospectively extracted from the outpatient clinic of the liver unit and from the Center for Health Promotion of Seoul National University Hospital from January 2001 to August 2003. These subjects were divided into two groups according to their serologic markers: the chronic carrier (CC) group and the spontaneously recovered (SR) group. The CC and SR groups were composed of 670 and 433 subjects, respectively (Table 1). The HBsAg-positive patients (CC group) were hepatitis B surface antigen (HBsAg)-positive over a 6-month period. They were followed up for disease progression at least every 6 months. The diagnoses of the CC and SR subjects were established by repeated seropositivity of the hepatitis B surface antigen (Enzygnost® HBsAg 5.0; Dade Behring, Marburg, Germany), anti-HBs (Enzygnost® Anti-HBs II) and anti-HBc (AB-Corek; DiaSorin s.r.l., Saluggia, Italy) of the IgG type without HBsAg, respectively, over a 6-month period. The CC group was further divided into two subgroups, i.e., those without (the CH/LC group; n=343) and those with HCC (the HCC group; n=327), according to the absence or presence of HCC, respectively. We excluded subjects who were positive for anti-HBs only and not for anti-HBc, as well as those who were positive for anti-HCV and anti-HIV (GENEDIA®; Greencross Life Science Corp., Yongin, Korea, HCV®3.2; Dong-A Pharmaceutical Co., Seoul, Korea). The patients who had other types of liver disease such as autoimmune hepatitis, toxic hepatitis, primary biliary cirrhosis, or Budd-Chiari syndrome were also excluded from the sample. None of the patients had a previous history of immunosuppression or anti-viral treatment.

Informed consent was gained from each patient, and the Institutional Review Board of Human Research at Seoul National University Hospital approved the study protocol. Liver cirrhosis was diagnosed pathologically or by the clinical evidences of portal hypertension such as visible collateral vessels on the abdominal wall, esophageal varices on the esophagogastroscopy, palpable splenomegaly, and sonographically definite findings of cirrhotic liver or ascites. HCC was diagnosed as described previously (Bruix *et al*., 2001). The clinical parameters are summarized in Table 1.

Table 1. Clinical profiles of study subjects

	SR	CC	
		CH or LC	HCC
No. of subjects	433	343	327
Age (mean (range))	54.9 (28～79)	49.8 (22～85)	58.3 (25～79)
Sex (male/female)	240/193	278/65	279/48
HBeAg (positive rate, %)	0	33.2	19.6
HBeAb (positive rate, %)	0	30	43.4
HBsAg (positive rate, %)	0	100	100
HBsAb (positive rate, %)	100	0	0.3
U albumin (positive rate, %)	0	7	13.5
U blood (positive rate, %)	28.2	12	22.9

SR, spontaneously recovered; CH, chronic hepatitis; CC, chronic carrier; LC, liver cirrhosis; HCC, hepatocellular carcinoma; U, Urine.

Sequencing analysis of *ITGB1*

Using the ABI PRISM 3730 DNA analyzer (Applied Biosystems, Foster City, CA), we sequenced all exons, including exon-intron boundaries and promoter regions (～1.5 kb), to discover polymorphisms of the *ITGB1* gene using DNA samples of 24 unrelated healthy Korean individuals. Twenty seven primer sets for amplification (Supplementary Table 1) and sequencing analysis were designed based on GenBank sequences (NT_008705.15). Sequence variants were verified by chromatograms.

Genotyping with fluorescence polarization detection

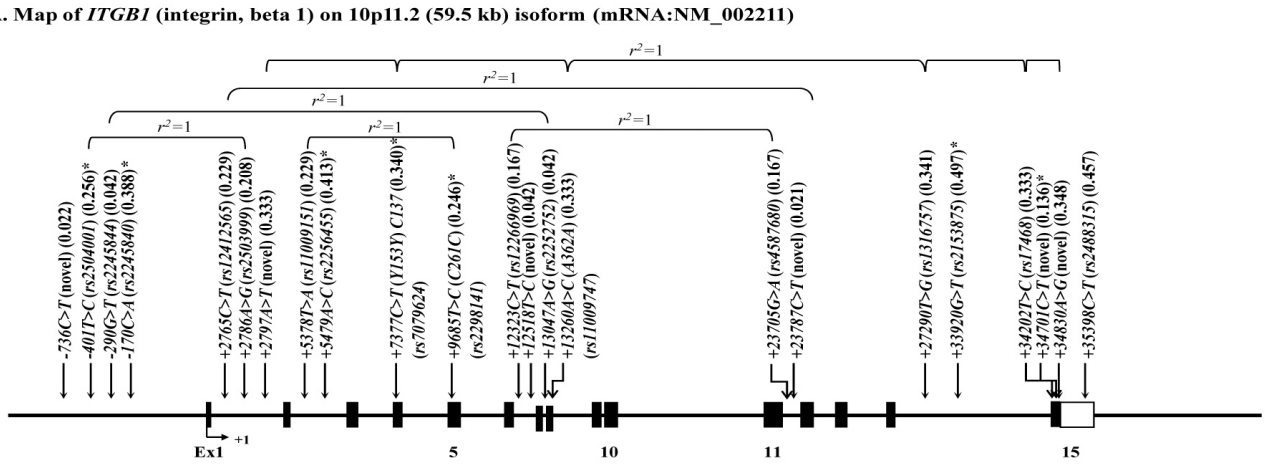
In order to genotype the polymorphic sites in our study, amplifying primers and probes were designed for TaqMan (Livak, 1999). Primer Express (Applied Biosystems) was used to design both the PCR primers and the MGB TaqMan probes. One allelic probe was labeled with the FAM dye and the other with the fluorescent VIC dye (Supplementary Table 2). Typically, PCR was run in the TaqMan Universal Master mix without UNG (Applied Biosystems) at a primer concentration of 900 nM and a

TaqMan MGB-probe concentration of 200 nM. The reaction was performed in a 384-well format in a total reaction volume of 5 ul 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). Primer sequences are listed in Supplementary Table 1.

Statistical analysis

Linkage disequilibrium (LD) was inferred using the algorithm (Haploview) that searches for a spine of strong |D' | and LD coefficient r^2 running from one marker to another (Barrett *et al*., 2005). Haplotypes of each individual were determined using the algorithm (PHASE, version 2.0) developed by Stephens *et al* (Stephens *et al*., 2001). Subjects with missing genotypes were omitted in the analysis of individual single-nucleotide polymorphisms (SNPs) and haplotypes. The genotyping success rate was > 99%, which makes the omission of a few individuals unlikely to change the results of the analysis. For analysis of viral clearance as an outcome, 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. Cox models were used for calculating relative hazards and P-values controlling for sex and status of liver cirrhosis among the CC group.

The effective number of independent marker loci in *ITGB1* was calculated to correct for multiple testing, using the software SNPSpD (http://genepi.qimr.edu.au/general/daleN/SNPSpD/), which is based on spectral decomposition (SpD) of matrices of pair-wise LD between SNPs (Nyholt, 2004). The resulting number of independent marker loci was applied to check for multiple testing. Statistical powers were calculated using the software, "Power for Genetic Association Analyses" (PGA) (Menashe *et al*., 2008). PGA is an application



B. Haplotypes in *ITGB1*

Hap.	-401T>C (rs2504001)	-170C>A (rs2245840)	+5479A>C (rs2256455)	+7377C>T (rs7079624)	+9685T>C (rs2298141)	+33920G>T (rs2153875)	+34701C>T (novel)	Freq.
ht1	T	A	A	T	T	C	G	0.284
ht2	C	C	C	C	T	C	T	0.219
ht3	T	C	A	C	C	C	T	0.214
ht4	T	C	C	C	T	C	G	0.125
ht5	T	A	A	T	T	T	G	0.054
Others	.	.	.	.	.	.	.	0.104

C. LDs among *ITGB1* polymorphisms

		|D'|						
		-401T>C (rs2504001)	-170C>A (rs2245840)	+5479A>C (rs2256455)	+7377C>T (rs7079624)	+9685T>C (rs2298141)	+33920G>T (rs2153875)	+34701C>T (novel)
	-401T>C (rs2504001)	-	1	0.996	1	1	0.02	0.971
	-170C>A (rs2245840)	0.18	-	1	1	1	0.042	0.997
	+5479A>C (rs2256455)	0.494	0.36	-	1	0.993	0.003	0.228
r^2	+7377C>T (rs7079624)	0.179	1	0.359	-	1	0.04	0.997
	+9685T>C (rs2298141)	0.115	0.17	0.228	0.171	-	0.103	0.964
	+33920G>T (rs2153875)	0	0.001	0	0.001	0.001	-	0.021
	+34701C>T (novel)	0.324	0.516	0.036	0.515	0.305	0	-

Fig. 1. Gene maps and haplotypes of the *ITGB1* gene. A. Polymorphisms identified in *ITGB1*. Coding exons are marked by shaded blocks and 3'-UTR by white blocks. Asterisks (*) indicate SNPs that were genotyped in the larger population. The LD coefficients (r^2) are based on the genotypes of Korean samples. B. Haplotypes of *ITGB1* in the Korean population. Only those with frequencies over 0.05 are shown. C. LD coefficients (|D'| and r^2) among the selected SNPs based on the genotypes of whole study subjects in this study (n=1,103).

specifically designed to calculate statistical power and other values in case-control association studies. A co-dominant (1df) model with relative risk 1.3, disease prevalence value of HBV 7.1% (Lee *et al.*, 1998), effective degree of freedom (EDF) 2, and an alpha error level of 5% was used to calculate the statistical power.

Results

Through direct sequencing of 24 individuals, we identified 23 sequence variants in the *ITGB1* gene: 4 in the promoter region, 7 in coding regions of exons, 11 in introns, and 1 in the 3'-untranslated region (3'-UTR) (Fig. 1A). Pairwise comparisons of all 23 polymorphisms revealed two sets of markers in absolute linkage disequilibrium (LD) (|D'|=1 and r^2=1, Fig. 1A). Among these 23 variants, 7 common polymorphisms (*−401T>C*, *−170C>A*, *+5479A>C*, *+7377C>T*, *+9685T>C*, *+33920G>T*, and *+34701C>T*) were selected for larger-scale genotyping (n=1,103) based on location, minor allele frequency (MAF>0.05), and LD. No significant deviations from Hardy-Weinberg equilibrium (HWE) were observed (p>0.05, Table 2). Five major haplotypes showed frequencies greater than 0.05 and accounted for over 89.6% of the distribution (Fig. 1B). Statistical powers of each polymorphism are shown in Table 3.

Association analyses of HBV clearance (CC vs. SR) and HCC occurrence (HCC vs. CH/LC) for each polymorphism and haplotype of the *ITGB1* gene were performed using logistic regression models, controlling for age and sex as covariates (Table 3). The P-values and odds ratios of each polymorphism and haplotype are shown in Table 3. Among polymorphisms, the second most common haplotype of the *ITGB1* gene, *ITGB1 haplotype-2 [C-C-C-C-T-C-T]*, was found to be putatively associated with HBV clearance, i.e., the frequency of individuals bearing the *ITGB1 haplotype-2* allele among the SR group was significantly higher than those in the CC group (freq.=0.248 vs. 0.199, OR=0.75, p=0.008, P^{corr}=0.05). Although the significances were not retained after correction for multiple testing, similar associations were also observed in *−401T>C* (*rs2504001*) and *+33920G>T* (*rs2153875*). There was no association observed between *ITGB1* polymorphisms or haplotypes and HCC occurrence (Table 3).

To analyze the role of *ITGB1* polymorphisms in the onset age of HCC, Cox relative hazards analysis for age of HCC occurrence was performed for the CC group. No significant association was observed (Table 3).

Discussion

Integrins play a major role in cell-cell and cell-ECM adhesion. They mediate signaling cascades that are involved in many cell functions. Up-regulated β integrins in the vascular endothelium function as angiogenesis inhibitors in blood vessels of some human tumors (Bridger *et al.*, 2008; Carlson *et al.*, 2008). The abnormal expression of β integrins and the deregulation of cell-ECM adhesion can be causes of tumor cell development, growth, and metastasis. Previous studies have suggested that the down-regulation of integrin $\alpha 5\beta 1$ is associated with the growth of tumor cells (Lara-Pezzi *et al.*, 2001; Bridger *et al.*, 2008), and higher expression of $\beta 1$ integrin (*ITGB1*) mRNA is significantly associated with docetaxel resistance, which inhibits the effect of chemotherapy for esophageal squamous cell carcinoma (Mori *et al.*, 2008). In addition, β integrins have key roles in the primary tumor formation, metastatic dissemination and inhibition of tumor cell senescence in mouse models which have breast and pancreatic cancer (Streuli and Akhtar, 2009).

In this study, we demonstrated that the second most common haplotype, *ITGB1 haplotype-2 [C-C-C-C-T-C-T]*, was putatively associated with HBV clearance. In addition, there were association signals in *−401T>C* (*rs2504001*) located in the promoter region and *+33920G>T* (*rs2153875*) in intron 14, although significances were not retained after correction for multiple testing. If the promoter polymorphism is located in transcription binding sites, it can induce the alteration of the transcription factor binding and affects transcriptional regulation. Previous studies demonstrated the associations of SNPs in promoter regions with HBV infection and/or HCC occurrence. First of all, histone deacetylase 10 (*HDAC10*) polymorphism (*HDAC10-589C>T*) was associated with the clearance of HBV infection and onset age of HCC. The promoter activity of this SNP was measured by luciferase activity reporter assay. The functional assay showed that luciferase activity of "T" allele was significantly higher than that of "C" allele of HDAC10-589C>T (Park *et al.*, 2007). Second, interleukin-18 (*IL-18*) polymorphism (*IL-18-148G>C*) in promoter is associated with the risk of HCC. The promoter activity of *IL-18-148G>C* measured by luciferase assay revealed that *−148C* allele represses transcriptional activity compared with the *−148G* allele (Kim *et al.*, 2009). In addition, the promoter SNPs of tumor necrosis factor-α (*TNF-α*) (*TNF-α-863* and *TNF-α-308*) (Kim *et al.*, 2003b) and transforming growth factor-$\beta 1$ (*TGF-β1*) (*TGF-β1-509*) were associated with HBV clearance and/or HCC occurrence (Kim *et al.*, 2003a).

The involvements of β integrin polymorphisms in HBV infection and HCC development have not been reported in previous studies. However, SNPs in other family member of integrin genes showed associations with

Table 2. Genotype and allele frequency of polymorphisms in ITGB1

Loci	Position	Amino acid change	rs#	Genotype				Frequency	Heterozygosity	HWE
−736C>T	Promoter		Novel	C	CT	T	N	0.022	0.043	0.915
				22	1	0	23			
−401T>C	**Promoter**	**-**	**rs2504001**	**T**	**CT**	**C**	**N**	**0.256**	**0.381**	**0.290**
				608	**401**	**78**	**1087**			
−290G>T	Promoter		rs2245844	G	GT	T	N	0.042	0.080	0.831
				22	2	0	24			
−170C>A	Promoter	-	rs2245840	C	AC	A	N	0.338	0.447	0.962
				474	487	123	1084			
+2765C>T	Intron1		rs12412565	C	CT	T	N	0.229	0.353	0.763
				14	9	1	24			
+2786A>G	Intron1		rs2503999	A	AG	G	N	0.208	0.330	0.236
				16	6	2	24			
+2797A>T	Intron1		Novel	A	AT	T	N	0.333	0.444	0.540
				10	12	2	24			
+5378T>A	Intron2		rs11009151	T	AT	A	N	0.229	0.353	0.763
				14	9	1	24			
+5479A>C	**Intron2**	**-**	**rs2256455**	**A**	**AC**	**C**	**N**	**0.413**	**0.485**	**0.140**
				371	**482**	**189**	**1042**			
+7377C>T	**Exon4**	**Tyr153Tyr**	**rs7079624**	**C**	**CT**	**T**	**N**	**0.340**	**0.449**	**0.828**
				470	**490**	**124**	**1084**			
+9685T>C	Exon5	Cys261Cys	rs2298141	T	CT	C	N	0.246	0.371	0.369
				622	391	71	1084			
+12323C>T	Intron6		rs12266969	C	CT	T	N	0.167	0.278	0.624
				17	6	1	24			
+12518T>C	Intron6		Novel	T	CT	C	N	0.042	0.080	0.000
				23	0	1	24			
+13047A>G	Intron7		rs2252752	A	AG	G	N	0.042	0.080	0.831
				22	2	0	24			
+13260A>C	Exon8	Ala362Ala	rs11009147	A	AC	C	N	0.333	0.444	0.540
				10	12	2	24			
+23705G>A	Intron11		rs4587680	G	AG	A	N	0.167	0.278	0.624
				17	6	1	24			
+23787C>T	Intron11		Novel	C	CT	T	N	0.021	0.041	0.917
				23	1	0	24			
+27290T>G	Intron14		rs1316757	T	GT	G	N	0.341	0.449	0.597
				9	11	2	22			
+33920G>T	**Intron14**	**-**	**rs2153875**	**G**	**GT**	**T**	**N**	**0.498**	**0.503**	**0.540**
				273	**521**	**268**	**1062**			
+34202T>C	Exon15		rs17468	T	CT	C	N	0.333	0.444	0.540
				10	12	2	24			
+34701C>T	**Exon15**	**-**	**Novel**	**C**	**CT**	**T**	**N**	**0.136**	**0.235**	**0.066**
				805	**270**	**13**	**1088**			
+34830A>G	Exon15		Novel	A	AG	G	N	0.348	0.454	0.472
				9	12	2	23			
+35398C>T	3'-UTR		rs2488315	C	CT	T	N	0.457	0.496	0.311
				8	9	6	23			

Bold-face indicates SNPs selected for larger-scale genotyping.

HBV and HCC. Integrin αv (*ITGAV*) SNPs, which are located in introns (*rs9333289, rs11685758,* and *rs2290083*) and 3'-UTR (*rs1839123*), and a haplotype (*haplotype-1 [C-C-G]*) were found to be significantly associated with chronic hepatitis and HBV-infected HCC in a Korean population (Lee *et al*., 2009). In that study, *rs9333289*, *rs11685758* and *rs1839123* were associated with susceptibility to HBV-infected HCC, and *rs2290083* was associated with susceptibility to both chronic infection of HBV and HBV-infected HCC. In addition, the major hap-

Table 3. Association analysis of ITGB1 SNPs and haplotypes with clearance of HBV infection, HCC occurrence and the onset age of HCC

Locus	Position	Amino acid	rs#	HBV clearance[a]						HCC occurrence[a]					Onset age of HCC[b]			
				CC (n=670)	SR (n=433)	OR (95%CI)	p	P^{corr*}	Statistical power (%)**	HCC (n=327)	CH/LC (n=343)	OR (95%CI)	p	Statistical power (%)**	n/event	χ^2	RH	p
−401T>C	Promoter	-	rs2504001	**0.240**	**0.281**	**0.80 (0.65～0.97)**	**0.03**	**0.16**	**98.7**	0.238	0.243	0.88 (0.65～1.20)	0.43	79.7	651/308	1.36	0.90	0.24
−170C>A	Promoter	-	rs2245840	0.344	0.329	1.11 (0.91～1.34)	0.31	-	99.7	0.353	0.338	1.11 (0.83～1.47)	0.48	92.2	648/305	0.50	1.06	0.48
+5479A>C	Intron2	-	rs2256455	0.405	0.423	0.92 (0.77～1.10)	0.36	-	99.9	0.383	0.427	0.84 (0.63～1.11)	0.22	95.5	608/292	0.81	0.93	0.37
+7377C>T	Exon4	Tyr153Tyr	rs7079624	0.349	0.328	1.14 (0.94～1.38)	0.17	-	99.7	0.357	0.343	1.09 (0.82～1.45)	0.55	92.4	649/308	0.23	1.04	0.63
+9685T>C	Exon5	Cys261Cys	rs2298141	0.245	0.248	0.95 (0.77～1.17)	0.62	-	98.4	0.256	0.231	1.12 (0.83～1.53)	0.46	80.3	650/308	0.05	1.02	0.83
+33920G>T	Intron14	-	rs2153875	**0.486**	**0.472**	**1.21 (1.01～1.45)**	**0.04**	**0.23**	**100.0**	0.488	0.480	1.04 (0.79～1.35)	0.79	97.7	627/293	0.67	1.07	0.41
+34701C>T	Exon15	-	Novel	0.137	0.134	1.10 (0.84～1.44)	0.51	-	85.2	0.134	0.142	1.00 (0.67～1.51)	0.99	53.4	652/315	0.11	1.04	0.74
ht1	-	-	-	0.288	0.277	1.07 (0.88～1.31)	0.50	-	99.2	0.300	0.278	1.11 (0.82～1.49)	0.51	86.7	634/303	0.36	1.06	0.55
ht2	-	-	-	**0.199**	**0.248**	**0.75 (0.61～0.93)**	**0.008**	**0.05**	**97.1**	0.195	0.202	0.92 (0.66～1.27)	0.61	71.1	634/303	0.26	0.95	0.61
ht3	-	-	-	0.214	0.214	0.96 (0.77～1.20)	0.73	-	96.9	0.215	0.210	0.96 (0.69～1.33)	0.81	74.1	634/303	0.55	0.93	0.46
ht4	-	-	-	0.134	0.112	1.28 (0.95～1.72)	0.10	0.62	81.9	0.119	0.148	0.85 (0.55～1.30)	0.44	51.7	634/303	0.01	0.99	0.94
ht5	-	-	-	0.054	0.053	1.10 (0.73～1.66)	0.64	-	42.8	0.055	0.054	1.22 (0.64～2.31)	0.55	21.4	634/303	0.26	1.09	0.61

[a]Logistic regression models were used for calculating odds ratios (95% confidential interval) and corresponding p-values for each SNP site and haplotypes controlling for age and sex ascovariates using SAS. Age (continuous value) and sex (male=0, female=1) were adjusted by including logistic analysis as covariables. All patients included in this study were HBsAg-positive (chronic hepatitis).

[b]Cox models were used for calculating relative hazards and P-values for SNPs and haplotypes, controlling for age, sex, adjusted age (age＜40, adjage=0; 40＜=age＜60, adjage=1; age〉60, adjage=2), LC (LC=0, no LC=1), and HBeAg (negative=0, positive=1) by SAS. All patients included in this table were HBsAg-positive (chronic HBV).

*To achieve the optimal correction for multiple testing of single nucleotide polymorphisms (SNPs) in linkage disequilibrium (LD) with each other, the effective number of independent marker loci (6.07) in ITGB1 was calculated using the software SNPSpD (http://genepi.qimr.edu.au/general/daleN/SNPSpD/), on the basis of spectral decomposition (SpD) of matrices of pair-wise LD between the SNPs.

**Statistical powers were calculated with OR=1.3 and alpha value=0.05, using the software, "Power for Genetic Association Analyses" (PGA).

Bold values indicate the case of $p < 0.05$

lotype, *haplotype-1 [C-C-G]* for *rs11685758-rs2290083-rs1839123*, was associated with decreased susceptibility to chronic infection of HBV (OR=0.59, 95% CI=0.36～0.97, p=0.04) and HBV-infected HCC (OR=0.58, 95% CI=0.34～0.98, p=0.04). In comparison with our study, the association between *haplotype-1 [C-C-G]* and the decreased susceptibility to chronic HBV infection was similar to our results with *ITGB1 haplotype-2 [C-C-C-C-T-C-T]*. The previous study may be less reliable than our study because of relatively small subjects (n=304) and p-values which did not undergo multiple testing corrections. However, in spite of the relatively less reliability, this study demonstrated the association between ITGAV polymorphisms and susceptibility to HBV infection (Lee *et al*., 2009). Therefore, the alpha subunit of integrin, ITGAV may be a candidate partner for the beta subunit of integrin, ITGB1 in the case of HBV infection.

Additional studies have suggested the associations between integrin polymorphisms in introns and human diseases. In particular, $\alpha 4$ and $\alpha 9$ integrin polymorphisms (*ITGA4* and *ITGA9*, respectively) were shown to be associated with autism and nasopharyngeal carcinoma (NPC), respectively. One *ITGA4* polymorphism in intron (*rs155100*) showed association with autism in a Portuguese population, and eight ITGA9 intron polymorphisms (*rs169188*, *rs197721*, *rs149816*, *rs169111*, *rs197770*, *rs2212020*, *rs189897*, and *rs197757*) were associated with NPC in a Malaysian Chinese population (Correia *et al*., 2009; Ng *et al*., 2009). Similar associations between integrin polymorphisms and human diseases in various European populations were proposed by another study, i.e., $\alpha 4$ integrin polymorphisms (*ITGA4*) (*rs1449263* in the promoter and *rs3770138* in an intron) were significantly associated with multiple sclerosis (MS) in Basque and Nordic populations (The *rs1449263* polymorphism was associated with MS only in the Basque population, whereas the *rs3770138* was associated only in the Nordic population.) (O'Doherty *et al*., 2007).

In summary, we identified 23 genetic variants in the human *ITGB1* gene. Seven common polymorphic sites were selected for genotyping in our HBV cohort, and statistical analyses showed that *ITGB1 haplotype-2 [C-C-C-C-T-C-T]* was putatively associated with HBV clearance. Our findings will provide useful information for further genetic studies of this important gene.

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Supplementary Table 1. Primer sequences for *ITGB1* SNP screening

	Forward	Reverse
Frag-1	TGATGCAGTTAGATTTCCCTGT	AACTACCCCAATTTCCTACCA
Frag-2	TCTTCTTGTGGAGGGACAGA	CACCTGAGGTCAGGAGTTTG
Frag-3	TGAGATGGAGTCCTGCTCTG	GAATCCATTCGTGCCTTTCT
Frag-4	CCGCAGGAGATAATGGAGAT	GCCTGACCATGAAGGAACTT
Frag-5	AGGGGATGAATACCCCATTT	CCACATCTGCCTAGTGGCTA
Frag-6	ACATCGGGTTGTTCAGCATA	TGCTGTGGTTGGATCTGAGT
Frag-7	TGCCCTCCAGATGACATAGA	ACAGGGCCACTCTACCATTC
Frag-8	TATCTTTGCATCCTGAAGGC	AGCATCCAGTGACAGAGGAA
Frag-9	CACCTTCCAAAGGAACCACT	ATGAATGCGCTTACTCCACA
Frag-10	CAGGATTTGGCTCATTTGTG	GCAATTTCAAGGTTCCTGGT
Frag-11	AGAGCTTCATTCAGTGGGCT	GGGAGTTGAGGCAAAGAGTG
Frag-12	AGAGCTTGTGGGTGACTGG	CTGCTGAAGTGAGTCGTGTG
Frag-13	TTGGCAAAGCATTCAAGTTC	TCCCACATTTCAATTCAGACA
Frag-14	TGCTCACCTTGTCCAGAAAC	TGGGCTCGCTAAAGTGTGTA
Frag-15	AAACCTGTTTCTCTGGCCTCTG	TGTCCCATTTCCTTCATGACAC
Frag-16	TTTCCATTGGAGATGAGGTATG	TTGTTGAATATACTTCTTTCATGGC
Frag-17	TGAACCCAGTACCTCAGTTGG	CCCTCAAGCTACCCCTTTTC
Frag-18	AACAATGGAGAGTGCGTCTG	GCTTAGGAGAGCCAAGAGGA
Frag-19	GATGTGTCAGACCTGCCTTG	GATTACAGGCACACACCACC
Frag-20	CACACCTGTAATCCCAGCAC	GGCTCTGCACTGAACACATT
Frag-21	TTCATTTATTGAAATCTTCCTTCA	TCAGGCATCGTTCTAAGTGTCT
Frag-22	TTGATCATGTATCCTGCAACCT	ACTGGCCACAGTAACCAGAA
Frag-23	AATAGCACCAGATAACGGGG	CAGAAACTCTCATCATGCTCATT
Frag-24	TGGTTTTACTCATGTGCAGG	TTCCCATGGCCTTTGTAGAT
Frag-25	TATAGCGATTGAAAGGGCAA	AGAGGTGACAGAAAGCACCA
Frag-26	AGAACCGAGCAATTTTCTGC	TGAACTACCCACCAACCAGA
Frag-27	GTGGCTATGCAACAGCTCTC	GATGAAACCTCTCTCCCAGC

Supplementary Table 2. Sequences of amplifying, Taqman probe, and extension primer for *ITGB1* SNP genotyping

Loci	rs#		Probe sequence
−401T>C	*rs2504001*	Forward	GCATTGAGGTAACTGAGGCATCTG
		Reverse	TCTCCTGCGGCTCCCA
		VIC	CCCAGACCAGCATAT
		FAM	CCAGACCGGCATAT
−170C>A	*rs2245840*	Forward	CGCAGTGTAGGTGCAAGGT
		Reverse	CAGACCCTCGCCCATCTC
		VIC	CCTCCCTAGCTGGTTC
		FAM	CCCTCCCTATCTGGTTC
+5479A>C	*rs2256455*	Forward	GATGTGATTTGATGTTTTGCAATTTAATTGCTT
		Reverse	CATTCCTTCCTGTAAAAATGTCTAAATGACA
		VIC	ACCATTACAATTTTCA
		FAM	ACCATTACACTTTTCA
+7377C>T	*rs7079624*	Forward	GAGAGCTGAAGACTATCCCATTGAC
		Reverse	TCTGTTCCAAGACTTTTTACATTCTCCAA
		VIC	TCTTTCATTGAGTAAGACAG
		FAM	TCTTTCATTGAATAAGACAG
+9685T>C	*rs2298141*	Forward	TTTCGATGCCATCATGCAAGTTG
		Reverse	TCCCCTGATAGGAAATGAATGCG
		VIC	CTTACTCCACAAACTG
		FAM	TTACTCCGCAAACTG
+33920G>T	*rs2153875*	Forward	AATAGCACCAGATAACGGGG
		Reverse	CAGAAACTCTCATCATGCTCATT
		Extension	ATTTTAGTAGGGTAACTGATAATTTTTCTCACTTTTTTTTTGTT
+34701C>T	Novel	Forward	CCTTCACTTTACAAATTCAAGCCTTAGA
		Reverse	TGGCCTGTATGTAAATAGTGCTAAATCAAG
		VIC	TTTTAGCAGAAAATTG
		FAM	TTTTAGCAAAAAATTG