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Identification of Polymorphisms in CYP2E1 Gene and Association Analysis among Chronic HBV Patients

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Abstract

Cytochrome P450 2E1 (CYP2E1) is a member of the cytochrome P450 superfamily, and it is a key enzyme responsible for the metabolic activation of many small-molecular-weight compounds such as alcohol, which is classified as a human carcinogen. In this study, we identified 19 single nucleotide polymorphisms (SNPs) in *CYP2E1* in Korean population. In these SNPs, we examined possible genetic association of *CYP2E1* polymorphisms with HBV clearance and the risk of hepatocellular carcinoma (HCC). Five common polymorphic sites were selected, *CYP2E1* polymorphisms at *rs3813867, rs3813870, rs2070673, rs2515641* and *rs2480257*, considering their allele frequencies, haplotype-tagging status and LDs for genotyping in larger-scale subjects (n=1,092). Statistical analysis demonstrated that *CYP2E1* polymorphisms and haplotypes show no significant association with HBV clearance, HCC occurrence and onset age of HCC ($p > 0.05$). Previous studies, however, have shown contradictory findings on associations of *CYP2E1* polymorphisms with *CYP2E1* activities and HCC risk. Comparing the contrasting results of previous researches suggest that CYP2E1 polymorphism is associated with *CYP2E1* activity induced by ethanol, but is not directly associated with HCC risk. *CYP2E1* variation/haploype information identified in this study will provide valuable information for future studies on *CYP2E1*.

Keywords: cytochrome P450 2E1 (CYP2E1), hepatocellular carcinoma (HCC), hepatitis B virus (HBV), chronic hepatitis (CH), liver cirrhosis (LC), polymorphism

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Introduction

The infection of hepatitis virus B (HBV) is considered as an important health problem worldwide with an estimation of 350 million people chronically infected with the disease (Lavanchy, 2005). Although hepatic complications do not develop in most chronic hepatitis B carriers, 15% to 40% will develop liver cirrhosis (LC) and hepatocellular carcinoma (HCC), which come with serious sequelae during their lifetime (Bosch *et al.*, 2005).

Continuous consumption of more than 80g of ethanol everyday for a period longer than 10 years increases the risk of HCC by approximately fivefold; however, consumption of less than 80g of alcohol per day does not significantly increases the risk of HCC (Morgan *et al.*, 2004). Although the development of alcohol related to HCC involves several factors including the presence of cirrhosis, oxidative stress, disturbed DNA methylation, and defective retinoic acid signaling, the precise mechanisms on how alcohol causes HCC at a molecular level are just beginning to emerge (Seitz & Stickel 2007; Stickel *et al.*, 2002). According to the International Agency for Research on Cancer, ethanol is classified as a human carcinogen because it induces HCC in animals and increases the risk for developing HCC in humans (Baan *et al.*, 2007; Seitz and Stickel 2007).

Cytochrome P450 2E1 (CYP2E1), a member of the cytochrome P450 superfamily is important for the metabolic activation of many low-molecular-weight toxicants such as N-nitrosamines, aniline, vinyl chloride, urethane and alcohol (Guengerich *et al.*, 1991). The participation of CYP2E1 in the ethanol metabolizing process is less important than alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). However, continuous ethanol consumption is known to increase the activity of CYP2E1 up to 20 fold, a major constituent of the microsomal ethanol oxidizing system in the liver (Stickel & Osterreicher 2006; Takahashi *et al.*, 1993). Therefore, alcohol' s metabolizing capacity is increased in heavy drinkers. Moreover, CYP2E1 often catalyze the metabolic activation of various procarcinogens to eventual carcinogens (Guengerich *et al.*, 1991; Koop, 1992).

Genetic variation appears to contribute to interindividual variation in CYP2E1 expression levels and activities (McCarver *et al.*, 1998). Specifically, RsaI polymorphism *(CYP2E1*5B) (rs2031920)* has been associated with decreased CYP2E1 activity or inducibility (Hayashi *et al.*, 1991; Lucas *et al.*, 1995; Marchand *et al.*, 1999;

Watanabe *et al.*, 1994). Functional *CYP2E1* polymorphisms might therefore influence on susceptibility to cancer development. In one study, common genotypes were associated with risk of HCC (Yu *et al.*, 1995). On the other hand, increased risk of HCC was observed with the rare genotype carriers in another study (Ladero *et al.*, 1996). These studies suggest that variation of *CYP2E1* can influence individual's risk of HCC.

Based on these studies, we have formed a hypothesis that polymorphisms of *CYP2E1* can affect HCC progression among HBV-infected patients and therefore performed a screening of *CYP2E1* to examine its genetic association with HBV clearance and HCC progression.

Methods

Subjects

A total number of 1,092 Korean subjects having either 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 from January 2001 to August 2003. All the study subjects were of Korean ethnicity. Subjects were classified into two different groups: CC (chronic carrier) and SR (spontaneously recovered), according to serological markers. The CC and SR cohorts consisted of 658 and 434 subjects, respectively, and the CC cohort was composed of 338 CH/LC and 320 HCC patients (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 six-month period, and for both anti-HBs (Enzygnost® Anti-HBs II; Dade Behring, Marburg, Germany) and anti-HBc (AB-Corek; DiaSorin s.r.l., Saluggia, Italy) of the IgG type without HBsAg, respectively. We excluded subjects that were positive only for anti-HBs and 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). Subjects whose average alcohol consumption assessed by interview was >510 g/day or average cigarette smoking was >1 pack/day were excluded. Patients who had any other types of liver diseases such as autoimmune hepatitis, toxic hepatitis, biliary cirrhosis and Budd-Chiari syndrome were also excluded. No patients in our study 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. All the patients in the CC group had been on regular medical follow-up and had been evaluated with serum alpha-fetoprotein level assessment, abdominal ultrasonography, and/or 2-phase spiral liver CT scan more than twice a year to detect early stages of HCC. We also performed abdominal MRI, bone scan, chest CT, brain MRI, brain CT, hepatic angiography or PET scan in some patients according to the clinical decisions. 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 esophagogastroscopy, palpable splenomegaly and sonographically definite findings of cirrhotic liver or ascites. HCC was diagnosed as described previously (the age of onset was determined by the date of the diagnosis.) (Bruix *et al.*, 2001).

Sequence analysis of the human *CYP2E1*

We have sequenced exons, including exon-intron boundaries and their flanking regions, including the promoter

Table 1. Clinical profiles of subjects

Profile	SR	CC	
		CH or LC	HCC
No. of subjects	434	338	320
Age (mean (range))	54.5 (22～79)	49.7 (22～81)	58.4 (24～79)
Sex (male/female)	243/191	274/64	273/47
HBeAg (positive rate, %)	0.2	25.8	14.5
HBeAb (positive rate, %)	0.9	23.5	32.3
HBsAg (positive rate, %)	0	77.9	73.7
HBsAb (positive rate, %)	100	0	0
U albumin (positive rate, %)	0	5.3	9.9
U blood (positive rate, %)	28.1	9.2	16.4

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

region (1.5 kb) to discover polymorphisms 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 *CYP2E1* were designed based on GenBank sequences (Ref. Genome seq.; NT_008818.16). Sequence variants were verified by chromatograms.

Genoyping with Fluorescence Polarization Detection

For genotyping of polymorphic sites in our HBV study, amplifying primers and probes were designed for TaqMan (Livak, 1999). One allelic probe was labeled with the FAM dye and the other with the fluorescent VIC dye for TaqMan probes. Information regarding the primers is attached in Table 2. Primer Express (Applied Biosystems, Foster City, CA) was used to design the MGB TaqMan probes. PCRs were run in a TaqMan Universal Master Mix without UNG (Applied Biosystems), with PCR primer concentrations of 900 nM and TaqMan MGB-probe concentrations of 200 nM. Reactions were performed in a 384-well format in a total reaction volume of 5 ml using 20 ng of genomic DNA. The plates were then placed in a thermal cycler (PE 9700, Applied Biosystems) and heated at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min with a final soak at 25°C. The TaqMan assay plates were transferred to the Prism 7900HT instrument (Applied Biosystems) where the fluorescence intensity in each well of the plate was read. Fluorescence data files from each plate were analyzed by automated allele-calling software (SDS 2.1).

Table 2. Sequences of amplifying and Taqman probe for *CYP2E1* SNP genotyping

rs#		Probe sequence
rs3813867	Forward	GCCAACGCCCCTTCTTG
	Reverse	TCATTGGTTGTGCTGCACCTA
	VIC	ACACTGCACCTCTCCT
	FAM	CACTGCAGCTCTCCT
rs3813870	Forward	TCTCTTCATTCTAACCACACACACAAA
	Reverse	ATTATTTTCTTCATACAGACCCTCTTCCAC
	VIC	CTATGGACTACCTTCATAGAA
	FAM	CTATGGACTACCTTCGTAGAA
rs2070673	Forward	GTTGTCTAACCAGTGCCAAAGG
	Reverse	TTGCCAACCCATAGTTAAGAACGT
	VIC	CAGGTCGGTACCTC
	FAM	CAGGACGGTACCTC
rs2515641	Forward	AGCCAGAACACTTCCTGAATGAAAA
	Reverse	CACCTGTGGAAAATGGCTTGAAAT
	VIC	CTGTACTTGAACTTTC
	FAM	ACTGTACTTAAACTTTC
rs2480257	Forward	GTGTGGAGGACACCCTGAAC
	Reverse	CAAAGAAAGGAATCAGTTTGAGAAATCCT
	VIC	CTTTCAAACAAGATTTCAA
	FAM	CTTTCAAACAAGTTTTCAA

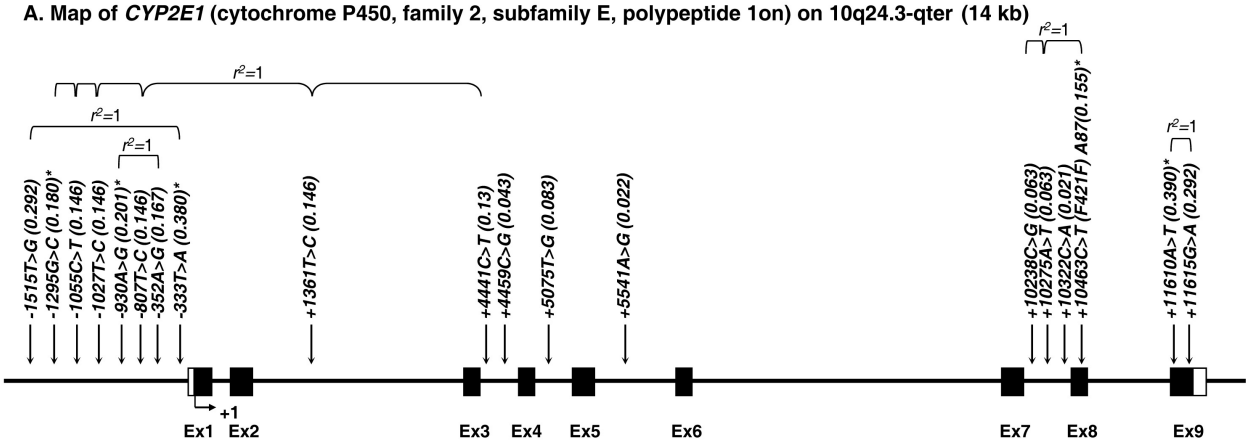
Statistics

We searched for a spine of strong |*D'*| and LD coefficient r^2 between all pairs of biallelic loci (Hedrick & Kumar, 2001). Linkage disequilibrium (LD) was inferred using the algorithm developed by the Broad Institute(using the program Haploview) (Barrett *et al*., 2005). Haplotypes of each individual were inferred using the algorithm (PHASE, version 2.0) developed by Stephens *et al*. (Stephens *et al*., 2001). Subjects harboring missing genotypes were omitted in the analysis of individual single-nucleotide polymorphisms (SNPs) and haplotypes. The genotyping success rate was >99%, so it is unlikely that omitting a small number of individuals introduced any bias in 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. HBV genotypes, HBV DNA and Alanine transaminase (ALT) levels have been regarded as important factors influencing HBV clearance and the development of HCC. However, HBV genotype C is predominant among CCs of the virus in Korea (Bae *et al*., 2005; Odgerel *et al*., 2003; Song *et al*., 2005a; Song *et al*., 2005b; Yoo *et al*., 2003), and the HBV DNA and ALT levels were found to be fluctuating during the follow-up for the majority of our HBV cohort. Therefore, logistic models for HBV clearance were adjusted only for age and sex. Statistical power is calculated with PGA (Power for Genetic Association Analyses) matlab application (http://dceg.cancer.gov/bb/tools/pga) (Menashe *et al*., 2008). PGA is an application specifically designed to calculate statistical power and other values of case-control genetic association studies. For the present study, a co-dominant (1df) model with relative risk 1.3, disease prevalence value 7.1% (Lee *et al*., 1998), EDF (Effective Degree of Freedom) 2, and alpha error level 5% were used to calculate the statistical power of p-values for HBV clearance and HCC occurrence.

Results

In this study, we examined the association of *CYP2E1* polymorphisms with persistent HBV infection and HCC occurrence. By direct sequencing of 24 individuals, we identified 19 sequence variants in the *CYP2E1*: 8 in the promoter, 3 in coding regions of exons (two non-synon-

A. Map of *CYP2E1* (cytochrome P450, family 2, subfamily E, polypeptide 1on) on 10q24.3-qter (14 kb)



B. Haplotypes in *CYP2E1*

Hap.	-1295G>C	-930A>G	-333T>A	+10463C>T	+11610A>T	Freq.
ht1	G	A	T	C	A	0.605
ht2	C	A	A	C	T	0.172
ht3	G	G	A	T	T	0.147
ht4	G	G	A	C	T	0.054
Others.	.	.	.	.	.	0.022

C. LDs among *CYP2E1* polymorphisms

		|D'|				
		-1295G>C	-930A>G	-333T>A	+10463C>T	+11610A>T
	-1295G>C	-	1	0.965	0.849	0.984
	-930A>G	0.056	-	1	0.963	0.991
r^2	-333T>A	0.339	0.412	-	0.981	0.985
	+10463C>T	0.029	0.657	0.282	-	0.980
	+11610A>T	0.335	0.389	0.929	0.268	-

Fig. 1. Gene maps and haplotypes of the *CYP2E1*. A. Polymorphisms identified in *CYP2E1*. Coding exons are marked by shaded blocks and 5' and 3'UTR by white blocks. The first base of the translational start site is denoted as nucleotide +1. Asterisks (*) indicate polymorphisms genotyped in a larger population (n=1,092). Polymorphisms identified in *CYP2E1* on chromosome 10q24.3-qter (Ref. Genome Seq. NT_008818.16). B. Haplotypes of *CYP2E1*. Only those with frequencies over 0.05 are shown. C. Linkage disequilibrium coefficients (|*D*'| and r^2) among selected SNPs based on the genotypes of whole study subjects in this study (n=1,092).

ymous and four synonymous) and 8 in introns (Fig. 1A, Table 3). We analyzed five polymorphisms (*rs3813867, rs3813870, rs2070673, rs2515641 and rs2480257*) in *CYP2E1*. These were selected for larger-scale genotyping (*n*=1,092) by considering their allele frequencies, haplotype-tagging status, and LDs. The minor allele frequencies of the SNPs in Korean population were; 0.181 (*rs3813867*), 0.202 (*rs3813870*), 0.381 (*rs2070673*), 0.157 (*rs2515641*) and 0.392 (*rs2480257*) (Fig. 1A, Table 3). Genotype distributions of all loci were in Hardy-Weinberg equilibrium ($p > 0.05$), except for *rs3813867* ($p < 0.05$, Table 3). Five SNPs showed low LDs (Fig. 1C), and four major haplotypes accounted for over 97.8% of the distribution (Fig. 1B).

Association analyses of HBV clearance (HCC/LC/CH vs. SR) and HCC occurrence (HCC vs. CH/LC) for each polymorphism and haplotype of the *CYP2E1* were performed using logistic regression models (Table 4), controlling age and sex as covariates (gender and age were significantly associated with HBV clearance; $p < 0.05$, data not shown) in our Korean HBV study. The statistical powers for each polymorphism are also shown in Table 4. In the analysis, *CYP2E1* polymorphisms and haplotypes did not show significant associations with HBV clearance and the HCC occurrence ($p > 0.05$, Table 4). The role of *CYP2E1* polymorphisms in onset age of HCC was also analyzed using Cox relative hazards model among chronic HBV patients (Table 4). However, no significant genetic effect was observed afterwards ($p > 0.05$, Table 4).

Discussion

In the present, HCC is one of the most common tumors among the prevalent malignant tumors worldwide (El-Serag & Mason, 2000). The increase in HCC occurrence

Table 3. Genotype and allele frequency of polymorphisms in *CYP2E1*

Loci	Position	Amino acid change	rs#	Genotype				Frequency	Heterozygosity	HWE[1]
−1515T>G	Promoter		*rs8192766*	T	GT	G	N	0.292	0.413	0.344
				13	8	3	24			
−1295G>C	**Promoter**		***rs3813867***	**G**	**GC**	**C**	**N**	**0.181**	**0.296**	**0.018**
				738	**298**	**47**	**1,083**			
−1055C>T	Promoter		*rs2031920*	C	CT	T	N	0.146	0.249	0.422
				18	5	1	24			
−1027T>C	Promoter		*rs2031921*	T	CT	C	N	0.146	0.249	0.422
				18	5	1	24			
−930A>G	**Promoter**		***rs3813870***	**A**	**AG**	**G**	**N**	**0.202**	**0.322**	**0.422**
				686	**337**	**48**	**1,071**			
−807T>C	Promoter		*rs2031922*	T	CT	C	N	0.146	0.249	0.422
				18	5	1	24			
−352A>G	Promoter		*rs2070672*	A	AG	G	N	0.167	0.278	0.396
				12	6	0	18			
−333T>A	**Promoter**		***rs2070673***	**T**	**TA**	**A**	**N**	**0.381**	**0.472**	**0.075**
				427	**482**	**171**	**1,080**			
1361T>C	Intron		*rs943975*	T	CT	C	N	0.146	0.249	0.422
				18	5	1	24			
4441C>T	Intron		*rs2070674*	C	CT	T	N	0.13	0.227	0.263
				18	4	1	23			
4459C>G	Intron		Novel	C	CG	G	N	0.043	0.083	0.827
				21	2	0	23			
5075T>G	Intron		*rs8192773*	T	GT	G	N	0.083	0.153	0.656
				20	4	0	24			
5541A>G	Intron		Novel	A	AG	G	N	0.022	0.043	0.915
				22	1	0	23			
10238C>G	Intron		*rs2070676*	C	CG	G	N	0.063	0.117	0.744
				21	3	0	24			
10275A>T	Intron		*rs2070677*	A	AT	T	N	0.063	0.117	0.744
				21	3	0	24			
10322C>A	Intron		Novel	C	AC	A	N	0.021	0.041	0.917
				23	1	0	24			
10463C>T	**Cds**	**Phe421Phe**	***rs2515641***	**C**	**CT**	**T**	**N**	**0.157**	**0.265**	**0.561**
				768	**291**	**24**	**1,083**			
11610A>T	**3'utr**		***rs2480257***	**A**	**AT**	**T**	**N**	**0.392**	**0.477**	**0.056**
				403	**471**	**176**	**1,050**			
11615G>A	3'utr		*rs2480256*	G	AG	A	N	0.292	0.413	0.967
				12	10	2	24			

[1]p-values of deviation from Hardy-Weinberg Equilibrium (HWE) among Korean subjects.
Bold face means SNPs genotyped in a larger population (n=1,092) and plain faces were based on the sequencing data (n=24).

is most likely due to the more widespread chronic infection with HBV. The virus-host interactions might influence different disease outcomes of HBV infections (Cruz *et al.*, 1987). However, knowledge of the host genetic factors involved in the progress of HBV infection and HCC occurrence is insufficient now. CYP2E1, an ethanol inducible enzyme, is one of the candidate genes that might influence the outcome of chronic liver disease. Moreover, acetaldehyde, a highly toxic and volatile compound is the first intermediate of alcohol metabolism by CYP2E1 (Peters & Ward, 1988). Therefore, the level of CYP2E1 might increase cancer risk.

Many researches have been focused on polymorphisms of the *CYP2E1*. There are many known polymorphic loci in the *CYP2E1*. Among these polymorphisms, RsaI polymorphism (*CYP2E1*5B*) (*rs2031920*) has been associated with higher transcription and increased enzyme activity and is located at 5' regulatory region (Grove *et al.*, 1998; Hayashi *et al.*, 1991; Tsutsumi *et al.*, 1994). RsaI polymorphism is associated with alcoholic liver disease in Asian population (Piao *et al.*, 2003; Tanaka *et al.*, 1997; Tsutsumi *et al.*, 1994). Likewise, some studies show that *CYP2E1* polymorphism were associated with risk of HCC (Ladero *et al.*, 1996; Yu *et*

Table 4. Logistic analysis of clearance of HBV infection, HCC occurrence and Cox relative hazards for age of HCC occurrence with SNPs and haplotypes

rs#	Position	Amino acid change	Clearance of HBV infection					HCC occurrence				Cox relative hazards			
			MAF					MAF							
			CC (n=658)	SR (n=434)	OR (95%CI)	p	Statistical power (%)*	HCC (n=320)	CH/LC (n=338)	OR (95%CI)	p	Statistical power (%)*	N/Event	χ^2	p	RH
rs3813867	Promoter	.	0.189	0.169	1.11 (0.89∼1.40)	0.36	86.8	0.192	0.186	1.05 (0.76∼1.47)	0.76	77.9	648/305	0.13	0.72	1.04
rs3813870	Promoter	.	0.202	0.202	1.02 (0.82∼1.28)	0.84	88.4	0.209	0.197	0.95 (0.69∼1.32)	0.77	80.5	641/304	0.01	0.92	1.01
rs2070673	Promoter	.	0.391	0.366	1.11 (0.92∼1.33)	0.28	96.7	0.400	0.384	0.96 (0.73∼1.25)	0.76	91.7	652/309	0.00	0.98	1.00
rs2515641	Exon8	Phe421Phe	0.160	0.152	1.06 (0.82∼1.36)	0.67	82.0	0.163	0.157	0.85 (0.59∼1.24)	0.4	72.4	649/308	0.90	0.34	0.90
rs2480257	Exon9	.	0.398	0.383	1.07 (0.89∼1.28)	0.49	96.8	0.411	0.387	1.01 (0.77∼1.33)	0.94	91.9	622/301	0.13	0.72	1.03
ht1		.	0.406	0.384	1.10 (0.92∼1.32)	0.31	96.9	0.413	0.401	0.97 (0.74∼1.26)	0.8	91.9	631/297	0.00	0.96	1.00
ht2		.	0.179	0.165	1.08 (0.86∼1.37)	0.50	85.3	0.181	0.176	0.99 (0.70∼1.40)	0.95	76.0	631/297	0.04	0.84	0.98
ht3		.	0.150	0.145	1.04 (0.81∼1.35)	0.76	79.8	0.156	0.145	0.91 (0.62∼1.33)	0.63	70.8	631/297	0.32	0.57	0.94
ht4		.	0.052	0.057	0.97 (0.65∼1.46)	0.89	38.0	0.054	0.050	1.10 (0.60∼2.03)	0.76	31.8	631/297	1.66	0.20	1.27

CC: chronic carrier group; SR = spontaneous recovered group.
Logistic regression models were used for calculating odds ratios (95% confidential interval) and corresponding P-values for each SNP sites and haplotypes controlling age, and sex as covariables using SAS. P-values of co-dominant model is also given. Age (continuous value) and sex (male=0, female=1) were adjusted by including in logistic analysis as co-variables. HBeAg (negative=0, blank=1, positive=2) for HCC occurrence were adjusted by inclusion in logistic analysis as covariates. All patients included in study were HBsAg-positive (chronic hepatitis).
*Statistical power of single associations was calculated with alpha error level of 5%, disease prevalence of 7.1%, EDF 2, given minor allele frequencies and sample sizes, and assuming a relative risk of 1.3, using PGA (Power for Genetic Association Analyses) software (http://dceg.cancer.gov/bb/tools/pga).

al., 1995). These studies further supports the polymorphism of CYP2E1 can influence to risk of HCC.

In this study, we hypothesized that polymorphisms of *CYP2E1* can affect HCC Risk in HBV patient. We analyzed five polymorphisms of *CYP2E1* via genotyping a total of 1,092 samples, including 658 chronic carrier and 434 spontaneously recovered individuals. However, our research revealed that the five polymorphisms and haplotypes in *CYP2E1* are not associated with HBV clearance, HCC occurrence and onset age of HCC in a Korean population.

Some of the previous studies have shown findings on *CYP2E1* polymorphism associations with CYP2E1 activities and HCC risk (Hayashi *et al*., 1991; Ladero *et al*., 1996; Lucas *et al*., 1995; Marchand *et al*., 1999; Watanabe *et al*., 1994; Yu *et al*., 1995). However, other previous studies have reported that there was no association between *CYP2E1* genetic polymorphisms and *CYP2E1* activities (Carriere *et al*., 1996; Inoue *et al*., 2000; Powell *et al*., 1998). In other words, including the findings of this study, the literature have shown contradictory findings on associations of *CYP2E1* polymorphisms with *CYP2E1* activities and HCC risk, especially rs3813867 polymorphism which is in absolute linkage disequilibrium (LD) ($|D'|$=1 and r^2=1) with RsaI polymorphism (*CYP2E1*5B*) (*rs2031920*), which is associated with higher transcription, increased enzyme activity, and alcoholic liver disease (Grove *et al*., 1998; Hayashi *et al*., 1991; Piao *et al*., 2003; Tanaka *et al*., 1997; Tsutsumi *et al*., 1994).

When comparing the contrasting results of previous researches, we can find different statistical methods and patient group in the researches. Positive result studies used a method of classification by physiological determinants, especially drinking and smoking habits. However, negative result studies only performed a case-control analysis without classifying according to alcohol consumption. Moreover, a previous study has shown that the activity of *CYP2E1* is modulated by various physiological determinants, such as obesity (O'Shea *et al*., 1994), fasting (O'Shea *et al*., 1994), and liver dysfunction (Dilger *et al*., 1997), and can be induced by ethanol (Girre *et al*., 1994). In contrast, dietary isothiocyanates (Leclercq *et al*., 1998), and garlic (Reicks and Crankshaw, 1996; Yang *et al*., 1994), as well as some drugs, such as disulfiram (Kharasch *et al*., 1993), and chlormethiazole (Gebhardt *et al*., 1997), inhibit *CYP2E1* activity. Therefore, we can estimate that RsaI polymorphism is not associated with *CYP2E1* activity, but rather, is associated with inducement of *CYP2E1* activity by gene-environment interactions, including links with ethanol consumption. Moreover, the increase of CYP2E1 activity may be associated with increase of alcoholic liver dis-

ease risk resulting in increased formation of ROS (Hayashi *et al*., 1991; Tsutsumi *et al*., 1994). This means that the findings from previous studies, which suggested that variation of *CYP2E1* can influence the risk of HCC, is due to *CYP2E1* activity change by ethanol consumption. In other words, we can presume that if the influence of environmental factors would have been be same, then there would be no association signals for the polymorphisms. We can presumption that *CYP2E1* polymorphism is associated with *CYP2E1* activity induced by ethanol, but is not directly associated with HCC risk. However, we can estimate another hypothesissuggesting that this contradictory result may be affected by racial differences. Additional studies are needed to clarify the association of *CYP2E1* with HCC risk in other ethnic populations.

In conclusion, this study presents that even if *CYP2E1* may have important functions in the HBV clearance and/or HCC occurrence, genetic variants of *CYP2E1* in this study probably do not influence HBV clearance and/or HCC occurrence. Although we could not find any significant associations, the information from this research would be useful for host genetic studies of *CYP2E1* related diseases, including HCC. Moreover, *CYP2E1* novel polymorphisms, which were identified in our research, will be useful for studies on alcohol related diseases.

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