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IGF2 polymorphisms are associated with hepatitis B virus clearance and hepatocellular carcinoma

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Abstract

The aim of this study was to determine whether *IGF2* polymorphisms are associated with the clearance of hepatitis B virus (HBV) infection and the risk of hepatocellular carcinoma (HCC). A total of 1095 Korean subjects were prospectively enrolled in this case-control study. The rates of *IGF2* polymorphisms were determined in each group. The *IGF2 + 820G* allele (*IGF2 + 820G/G*) and the *IGF2 + 6815A/A* genotype were strongly associated with the resolution of HBV infection (OR = 0.62–0.73; $P = 0.001$–0.03 and OR = 0.71; $P = 0.03$, respectively). Haplotype analysis showed that *IGF2-haplotype5* (A-C-C-T-A-T-G) and *IGF2-haplotype1* (T-C-T-T-A-C-A) were significantly associated with the clearance and persistence of HBV infection (OR = 0.55–0.58, $P = 0.009$–0.01 and OR = 1.31–1.65, $P = 0.001$–0.007, respectively). On the other hand, the *IGF2 + 2482C/C* or *+820G/G* genotypes were significantly associated with a higher risk of HCC (OR = 1.88, 1.68; $P = 0.04$). *IGF2* polymorphisms were found to be strongly associated with the clearance of HBV or the occurrence of HCC in patients with chronic HBV infection.

Keywords: *IGF2*; SNPs; Polymorphisms; Hepatitis B virus infection; Hepatocellular carcinoma; Haplotype; Association study

Hepatitis B virus (HBV) infection is a global public health problem, as HBV infects more than 350 million people worldwide [1]. 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 (HCC). Age at infection is a well-known major determinant of progression to chronicity; chronic infection occurs in approximately 90% of infants infected at birth, in 25–50% of children infected between the ages of 1 and 5 years, and in less than 5% of those infected during adult life [2–4]. However, the mechanisms underlying the resolution of acute HBV infection and 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 an HBV endemic area like Korea, it is apparent that the outcome of the infection does not appear to be determined by variations in the virulences of viral strains [5,6], but that rather host factors are more likely to influence disease outcome [7,8]. In addition, it was previously demonstrated that genotype C HBV predominates (almost all) among chronic carriers of the virus in Korea [9–11]. Thus, it is conceivable that genetic differences play an important role. Furthermore, among patients with chronic HBV infection, family history is a known risk factor for the development of HCC [12]; therefore, genetic factors are also likely to modify the risk of developing HCC. Indeed, we and other researchers have previously reported that several genetic polymorphisms of tumor necrosis factor-α, interleukin 10, transforming growth factor-β1, cytotoxic T-lymphocyte antigen 4, and mannose binding lectin might influence the

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natural course of chronic HBV infections [13–18]. However, each genetic polymorphism previously associated with HBV confers a low effect (odds ratio [OR], 0.52–3.08). Therefore, the genetic polymorphisms associated with HBV clearance and progression to HCC warrant further study.

Cell-mediated immune responses directed toward infected liver cells have been considered to be the main inducers of hepatic injury and mediators of HBV clearance [7,19]. Moreover, of the several cytokines involved in cell mediated responses, insulin-like growth factor (IGF)-II is known to have a stimulating effect on T-lymphocyte proliferation, which is mediated by the binding of IGF-II to its receptor [20]. IGF-II, one of the families of growth-hormone-dependent proteins, also plays key roles in mitogenic activity, anti-apoptosis, and in immune system regulation. The capacity of an individual to produce cytokines is largely determined genetically, and individuals differ markedly in this context. Moreover, these differences have been ascribed to polymorphisms within the regulatory regions or signal sequences of cytokine genes [21]. Several biallelic polymorphisms have been described within the *IGF2* gene [22], and a number of studies have shown that the *IGF2* polymorphism has a significant effect on transcriptional activity [23].

Thus, we postulated that *IGF2* polymorphisms may be associated with hepatocarcinogenesis as well as HBV clearance [24]. Here, we compared the prevalences of polymorphisms associated with the *IGF2* gene in subjects with chronic HBV infection (chronic carrier; CC group) with those of subjects with serologic evidence of spontaneous recovery (SR group), and in subjects with or without HCC to determine whether polymorphisms of the *IGF2* gene are associated with HCC occurrence.

Materials and methods

Study subjects. A total of 1095 Korean subjects with either present or past evidence of HBV infection were prospectively enrolled at the outpatient clinic of the liver unit or at the Center for Health Promotion of Seoul National University Hospital between January 2001 and August 2003. Subjects were placed in 2 groups: the CC ($n = 666$) or the SR ($n = 429$) groups, according to serologic markers (Table 1). Diagnoses of CC and SR subjects were established by repeated testing for the seropositivity of 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; DiaSorin s.r.l., Saluggia, Italy) of the IgG type without HBsAg, respectively. 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 [25]. We excluded subjects who were positive for anti-HBs alone without 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). Patients with other types of liver diseases, such as, autoimmune hepatitis, toxic hepatitis, primary biliary cirrhosis, or Budd-Chiari syndrome were also excluded. No patient had a previous history of immunosuppression or anti-viral treatment. Informed consent was obtained from all patients, and the Institutional Review Board of Human Research at Seoul National University Hospital approved the study protocol. Clinical parameters are summarized in Table 1.

Genotyping using fluorescence polarization detection. For genotyping polymorphic sites, amplifying primers and probes were designed for TaqMan [26]. Primer Express (Applied Biosystems) was used to design both the PCR primers and the MGB TaqMan probes. The GenBank Accession Nos. for the IGF2 gene studied in this article are L15440, Y13633, and X07868. Information regarding the primers used is available on our website (http://www.snp-genetics.com/reference/supplementatry information to IGF2_HBV.doc). One allelic probe was labeled with FAM dye and the other with fluorescent VIC dye. PCRs were run in a TaqMan Universal Master mix without UNG (Applied Biosystems) using PCR primer concentrations of 900 nM and TaqMan MGB-probe concentrations of 200 nM. Reactions were performed in a 384-well format in total reaction volumes of 5 μl using 20 ng of genomic DNA. Plates then were placed in a thermal cycler (PE 9700, Applied Biosystems), heated at 50 °C for 2 min, 95 °C for 10 min, and subjected to 40 amplification cycles of 95 °C for 15 s and 60 °C for 1 min. The TaqMan assay plates were then transferred to a Prism 7900HT instrument (Applied Biosystems) and the fluorescence intensities of wells were read. Plate fluorescence data files were analyzed using automated software (SDS 2.1).

Statistics. The χ^2 tests were used to determine whether individual variants were in equilibrium at each locus in the population (Hardy–Weinberg equilibrium). We examined widely used measures of linkage disequilibrium between all pairs of biallelic loci Lewontin's D′ (|D′|) [27] and r^2. Haplotypes and their frequencies were inferred using the algorithm developed by Stephens et al. [28]. Genotypes were given codes of 0, 1, and 2; 0, 1, and 1; or 0, 0, and 1 in codominant, dominant, and recessive models, respectively. Logistic regression analysis was used to calculate odds ratios (95% confidential interval) and corresponding *P* values, whilst controlling for age and sex as covariates.

Table 1
Clinical characteristics of the study subjects

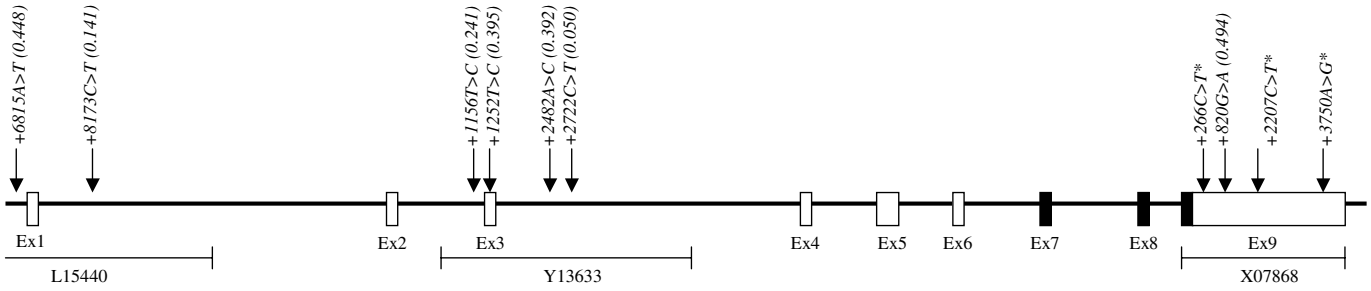
Profile	SR	CC	
		CH or LC	HCC
No. of subjects	429	339	327
Age (mean (range))	54.9 (28–79)	49.9 (22–85)	58.3 (25–79)
Sex (male/female)	240/189	274/65	277/50
HBeAg (positive rate, %)	0	33.5	19.6
HBeAb (positive rate, %)	38.2	47.3	63.8
HBsAg (positive rate, %)	0	100	100
HBsAb (positive rate, %)	100	0	0

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

Results

The locations of the ten known polymorphisms and the distributions of rare alleles containing seven of these ten polymorphisms are shown in Fig. 1A and Table 2. Three polymorphisms, including $+266C > T$, $+2207C > T$, and $+3750G > A$, were found to occur as homozygotes in Korean population studied. No significant deviations from the Hardy–Weinberg equilibrium were observed for all polymorphisms ($P > 0.05$). Five major haplotypes accounted for over 90% of distribution, and *IGF2-ht1*, *-ht3*, and *-ht6* were almost equivalent to $+820A > G$, $+8173C > T$, and $+2722C > T$, respectively (Fig. 1B). Linkage disequilibrium coefficients (|D′|) and r^2 among polymorphisms were also calculated (Fig. 1C). Haplotypes with either equivalence

A **Map of Insulin-like growth factor-2 (IGF2) on chromosome 11p15.5**



B **Haplotype of *IGF2* polymorphisms**

Hap.	+6815T>A	+8173C>T	+1156T>C	+1252T>C	+2482A>C	+2722C>T	+820A>G	Freq.
ht1	T	C	T	T	A	C	A	0.467
ht2	A	C	C	C	C	C	G	0.168
ht3	A	T	T	C	C	C	G	0.146
ht4	A	C	T	C	C	C	G	0.060
ht5	T	C	T	T	A	C	G	0.050
ht6	A	C	C	T	A	T	G	0.040
Others(1)	.	.	.	.	.	.	.	0.068

C **LDs among *IGF2* polymorphisms**

		|D'|						
		+6815T>A	+8173C>T	+1156T>C	+1252T>C	+2482A>C	+2722C>T	+820A>G
	+6815T>A	-	1	0.865	0.930	0.932	0.794	0.831
	+8173C>T	0.226	-	0.895	0.951	0.946	0.938	0.863
	+1156T>C	0.280	0.050	-	0.646	0.639	0.987	0.871
r^2	+1252T>C	0.700	0.253	0.193	-	0.996	1	0.909
	+2482A>C	0.702	0.251	0.189	0.989	-	1	0.911
	+2722C>T	0.040	0.009	0.164	0.037	0.037	-	0.802
	+820A>G	0.619	0.151	0.254	0.599	0.601	0.036	-

Fig. 1. Gene maps and haplotypes of the *IGF2* gene. (A) Polymorphisms identified in *IGF2*. Coding exons are marked by shaded blocks and 5′ and 3′UTR by white blocks. Bold face type indicates SNPs genotyped in the larger population, and asterisks (*) indicate homozygotes. All SNPs are described in detail by Gaunt et al. [22]. (B) Haplotypes of *IGF2* in the Korean population. Only those with frequencies over 0.03 are shown. Others (1) contain rare haplotypes: ACTTACA, TCCCCCG, ACCCCCA, ATTCCCA, TCCTATG, ACCTATA, ATTTACA, ATCCCCG, TCTCCCG, TCCCCCA, ACTTACG, TCCCACA, ATTCACG, ACTCCCA, ACTTCCA, TCTTCCG, ATCTAT, and GCTTATA. (C) LD coefficients (|D′| and r^2) among SNPs in *IGF2*.

Table 2
Genotype distributions of *IGF2* polymorphisms in Korean population ($n = 1095$)

Locus	Position	Accession No.	rsSNP	Frequency		HWE[b]	Heterozygosity[c]
				This study (Korean, $n = 1,095$)	Gaunt et al. (Caucasian, $n = 2560$)		
+6815T > A	Promoter	L15440	rs3842759	0.46	0.24[a]	0.997	0.495
+8173C > T	Intron1	L15440	rs10840452	0.16	0.34[a]	0.894	0.242
+1156T > C	Intron2	Y13633	rs3741211	0.24	0.48[a]	0.916	0.366
+1252T > C	Exon3 (5′UTR)	Y13633	rs10770125	0.41	0.47[a]	0.363	0.478
+2482A > C	Intron3	Y13633	rs1003483	0.41	0.48[a]	0.443	0.476
+2722C > T	Intron3	Y13633	—	0.05	0.28[a]	0.546	0.094
+266C > T	3′UTR	X07868	rs2230949	0	0.10[a]	—	—
+820A > G	3′UTR	X07868	rs680	0.50	0.72[a]	0.957	0.5
+2207C > T	3′UTR	X07868	—	0	0.05[a]	—	—
+3750G > A	3′UTR	X07868	—	0	0.89[a]	—	—

[a] $P < 0.0001$; P values for the difference of distribution of polymorphisms between Korean and Caucasian.
[b] P values of deviation from Hardy–Weinberg equilibrium in the Korean population.
[c] Heterozygosity calculated in the Korean population.

with single polymorphisms or with frequencies of less than 3% were excluded from further analysis.

In the initial analysis, the *IGF2 + 820G* allele (*IGF2 + 820G/G*) showed a significant association with the clearance of HBV infection (OR = 0.62–0.73, $P = 0.001$–0.03; Table 3), whereas other loci showed no significant associations, although a marginal association between *+6815T > A* and HBV clearance was observed (OR = 0.71, $P = 0.03$ in recessive model). In further haplotype analysis, one common haplotype, *IGF2-haplotype5* (A-C-C-T-A-T-G), was found to be significantly associated with HBV clearance, and *IGF2-haplotype1* (T-C-T-T-A-C-A) was significantly associated with HBV persistence (OR = 0.55–0.58, $P = 0.009$–0.01 and OR = 1.31–1.65, $P = 0.001$–0.007, respectively; Table 3).

Table 3
Logistic analysis of clearance of HBV infection for *IGF2* SNPs and haplotypes

Locus	Genotype	CC	SR	Codominant		Dominant		Recessive	
				OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
+6815T > A	*TT*	**192(29.6%)**	**122(28.4%)**						
	AT	**334(51.5%)**	**203(47.3%)**	0.89(0.75–1.07)	0.23	1.02(0.77–1.35)	0.92	**0.71(0.52–0.96)**	**0.03**
	AA	**123(19.0%)**	**104(24.2%)**						
+8173C > T	*CC*	457(71.1%)	295(68.8%)						
	CT	174(27.1%)	126(29.4%)	0.91(0.71–1.17)	0.46	0.90(0.68–1.18)	0.44	0.94(0.37–2.41)	0.90
	TT	12(1.9%)	8(1.9%)						
+1156T > C	*TT*	369(57.8%)	244(57.3%)						
	CT	240(37.6%)	154(36.2%)	0.92(0.74–1.14)	0.45	0.97(0.75–1.26)	0.81	0.66(0.38–1.16)	0.15
	CC	29(4.6%)	28(6.6%)						
+1252T > C	*TT*	229(36.2%)	136(31.9%)						
	CT	306(48.4%)	215(50.4%)	0.86(0.72–1.04)	0.12	0.84(0.64–1.10)	0.21	0.80(0.57–1.13)	0.21
	CC	97(15.4%)	76(17.8%)						
+2482A > C	*AA*	201(35.1%)	132(31.8%)						
	AC	288(50.3%)	210(50.6%)	0.88(0.72–1.07)	0.19	0.89(0.67–1.18)	0.41	0.77(0.54–1.11)	0.16
	CC	84(14.7%)	73(17.6%)						
+2722C > T	*CC*	586(89.6%)	394(91.8%)						
	CT	65(9.9%)	32(7.5%)	1.23(0.82–1.85)	0.33	1.30(0.83–2.04)	0.24	0.74(0.14–3.92)	0.72
	TT	3(0.5%)	3(0.7%)						
+820A > G	*AA*	**174(27.8%)**	**96(22.4%)**						
	AG	**329(52.6%)**	**219(51.2%)**	**0.73(0.61–0.89)**	**0.001**	**0.71(0.53–0.97)**	**0.03**	**0.62(0.46–0.84)**	**0.002**
	GG	**122(19.5%)**	**113(26.4%)**						
ht1	*–/–*	**132(23.6%)**	**130(31.9%)**						
	–/ht1	**303(54.2%)**	**203(49.8%)**	**1.31(1.08–1.60)**	**0.007**	**1.65(1.22–2.22)**	**0.001**	1.19(0.85–1.67)	0.30
	ht1/ht1	**124(22.2%)**	**75(18.4%)**						
ht2	*–/–*	382(68.3%)	287(70.3%)						
	–/ht2	164(29.3%)	107(26.2%)	1.04(0.81–1.34)	0.77	1.12(0.83–1.49)	0.46	0.63(0.28–1.40)	0.26
	ht2/ht2	13(2.3%)	14(3.4%)						
ht4	*–/–*	500(89.5%)	353(86.5%)						
	–/ht4	58(10.4%)	54(13.2%)	0.77(0.52–1.15)	0.20	0.77(0.52–1.15)	0.20	0.55(0.03–8.85)	0.67
	ht4/ht4	1(0.2%)	1(0.3%)						
ht5	*–/–*	**515(92.1%)**	**360(88.2%)**						
	–/ht5	**42(7.5%)**	**46(11.3%)**	**0.58(0.38–0.88)**	**0.01**	**0.55(0.35–0.86)**	**0.009**	0.62(0.08–4.81)	0.65
	ht5/ht5	**2(0.4%)**	**2(0.5%)**						

Logistic regression models were used for calculating odds ratios (95% confidential interval) and the corresponding *P* values of codominant model for SNP sites and haplotypes, controlling age and sex as covariates. *IGF2-ht1* and *-ht3* were almost equivalent to *+820A > G* and *+8173C > T*, respectively. Haplotypes with either equivalence single polymorphisms or with frequencies less than 3% were excluded from the statistical analysis.

Association analysis of *IGF2* polymorphisms with HCC occurrence among patients with a chronic HBV infection revealed that $IGF2 + 820A > G$ and $IGF2 + 2722A > C$ showed susceptible effect on the HCC occurrence in recessive analysis model (OR = 1.68, $P = 0.04$ and OR = 1.88, $P = 0.04$, respectively; Table 4). Similarly, by Cox analysis of age to HCC, $IGF2 + 820A > G$ also showed a susceptible effect in codominant and recessive models (RH = 1.23–1.39, $P = 0.02$; Table 5). Acceleration to HCC occurrence was demonstrated in $IGF2 + 820G/G$ bearing patients as compared with heterozygous $IGF2 + 820A/G$ and wild type by Kaplan-Meier survival curve analysis ($P = 0.02$; Fig. 2).

Discussion

The present study shows that the genetic polymorphisms and the haplotypes of *IGF2* are associated with the clearance of HBV and the occurrence of HCC; $IGF2 + 820G$ allele ($IGF2 + 820G/G$) and *IGF2-haplotype5* (A-C-C-T-A-T-G) were significantly associated with the clearance of HBV infection (OR = 0.62–0.73, $P = 0.001$–0.03 and OR = 0.55–0.58, $P = 0.009$–0.01, respectively). In contrast, *IGF2-haplotype1* (T-C-T-T-A-C-A) was significantly associated with the persistence of HBV infection (OR = 1.31–1.65, $P = 0.001$–0.007); $IGF2 + 820A > G$ and $IGF2 + 2722A > C$ were associated with a susceptibility to HCC occurrence by recessive analysis model (OR = 1.68, $P = 0.04$ and OR = 1.88, $P = 0.04$, respectively).

The frequencies of the less frequent alleles in the present study differed significantly from those reported in Caucasians ($P < 0.0001$ for all polymorphisms) [22]. Differences between the allele frequencies of polymorphisms of the *IGF2* gene in Caucasians and Koreans might be explained by ethnic differences. Lee et al. previously screened for 300 single nucleotide polymorphisms (SNPs), selected from the

Table 4
Analysis of HCC occurrence in CC with *IGF2* SNPs and haplotypes

Gene	Locus	HCC ($n = 314$)	CH/LC ($n = 335$)	Codominant		Dominant		Recessive	
				OR(95% CI)	*P*	OR(95% CI)	*P*	OR(95% CI)	*P*
IGF2	*+6815T > A*	0.470	0.427	1.06(0.81–1.40)	0.66	0.88(0.58–1.33)	0.53	1.45(0.89–2.35)	0.13
	+8173C > T	0.168	0.140	1.21(0.83–1.77)	0.32	1.12(0.74–1.70)	0.60	4.34(0.95–19.79)	0.06
	+1156T > C	0.244	0.224	1.10(0.79–1.51)	0.58	1.09(0.74–1.60)	0.67	1.28(0.53–3.11)	0.59
	+1252T > C	0.417	0.375	1.07(0.81–1.42)	0.62	0.89(0.59–1.33)	0.56	1.60(0.93–2.75)	0.09
	+2482A > C	**0.415**	**0.382**	1.09(0.80–1.47)	0.59	0.84(0.55–1.30)	0.44	**1.88(1.03–3.44)**	**0.04**
	+2722C > T	0.051	0.058	0.80(0.45–1.42)	0.45	0.88(0.48–1.63)	0.69	0.09(0.01–1.25)	0.07
	+820A > G	**0.482**	**0.435**	1.15(0.86–1.53)	0.35	0.91(0.58–1.42)	0.67	**1.68(1.02–2.75)**	**0.04**
	ht1	0.480	0.504	1.00(0.74–1.36)	1.00	0.65(0.40–1.06)	0.09	1.60(0.96–2.67)	0.07
	ht2	0.179	0.160	1.07(0.72–1.59)	0.75	1.09(0.70–1.70)	0.71	0.97(0.25–3.75)	0.96
	ht4	0.050	0.059	0.72(0.38–1.37)	0.31	0.72(0.38–1.37)	0.31	—	0.98
	ht5	0.039	0.042	0.82(0.41–1.64)	0.58	0.80(0.38–1.68)	0.55	0.97(0.06–17.07)	0.98

Logistic regression models were used for calculating odds ratios (95% confidential interval) and corresponding *P* values for each SNP sites and haplotypes controlling age, sex and the presence of liver cirrhosis (LC) as covariables using SAS. The *P* values of codominant, dominant and recessive models are also given. Age (continuous value), sex (male = 0, female = 1), LC (LC = 1, no LC = 0) and HBeAg (negative = 0, blank = 1, positive = 2) were adjusted for in the logistic analysis as co-variables. All patients included in the study were HBsAg-positive (chronic hepatitis; CH).

Table 5
Cox relative hazards analysis for age of HCC occurrence as a function of *IGF2* SNPs and haplotypes

Gene	Locus	*n*/event	Codominant			*n*/event	Dominant			*n*/event	Recessive		
			χ^2	RH	*P*		χ^2	RH	*P*		χ^2	RH	*P*
IGF2	*+6815T > A*	642/307	0.74	1.08	0.39	642/307	0.05	1.03	0.82	642/307	1.47	1.19	0.23
	+8173C > T	636/305	0.44	1.08	0.50	636/305	0.18	1.06	0.67	636/305	1.04	1.46	0.31
	+1156T > C	631/301	1.46	1.13	0.23	631/301	1.42	1.16	0.23	631/301	0.35	1.17	0.56
	+1252T > C	625/301	1.40	1.11	0.24	625/301	0.12	1.04	0.73	625/301	3.16	1.31	0.08
	+2482A > C	566/280	1.25	1.11	0.26	566/280	0.04	1.03	0.84	566/280	3.42	1.34	0.06
	+2722C > T	647/305	0.02	0.97	0.88	647/305	0.07	1.05	0.79	647/305	1.44	0.30	0.23
	+820A > G	**618/301**	**5.69**	**1.23**	**0.02**	618/301	2.56	1.25	0.11	**618/301**	**5.44**	**1.39**	**0.02**
	ht1	552/273	0.89	0.92	0.34	552/273	2.99	0.78	0.08	552/273	0.05	1.04	0.82
	ht2	552/273	0.79	1.11	0.38	552/273	0.95	1.14	0.33	552/273	0.01	1.05	0.91
	ht4	552/273	0.04	0.96	0.84	552/273	0.04	0.96	0.84	552/273	0.00	0.00	0.98
	ht5	552/273	0.18	1.10	0.67	552/273	0.11	1.08	0.74	552/273	0.35	1.82	0.56

Cox models were used to calculate the relative hazards and *P*-values for SNPs and haplotypes controlling sex, adjusted-age (age < 40, Adjage = 0; 40 <= age < 60, adjage = 1; age < 60, adjage = 2), LC (LC = 0; noLC = 1) and HBeAg (negative = 0, blank = 1, positive = 2) by SAS.

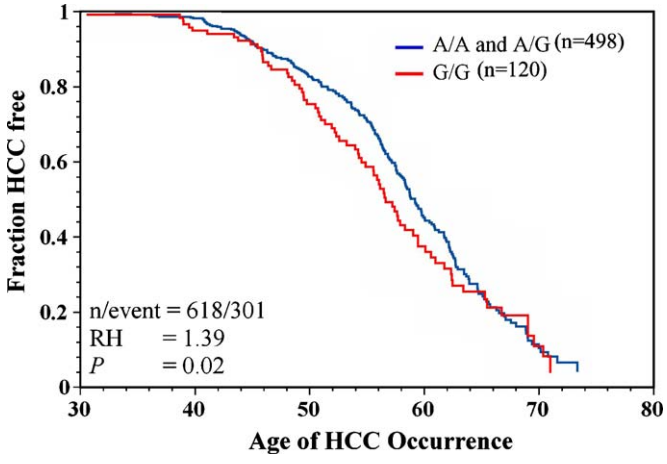


Fig. 2. Kaplan–Meyer survival curves demonstrating age of hepatocellular carcinoma (HCC) occurrence based on *IGF2 + 820A > G*. Number of patients (*n*), *P* values and relative hazards based on Cox proportional hazards model are given.

public database, in 24 Korean individuals and found that approximately 23% of the SNPs were not found [29].

It is possible that the SNPs that we found to be associated with hepatitis B clearance or the risk of HCC may be related with circulating IGF-II levels. The liver is the main source of plasma IGFs released into the circulation. In randomly selected *IGF2 + 820A/G* homozygotes, IGF-II levels were significantly higher in *+820A/A* homozygotes than in *+820G/G* homozygotes [30], suggesting that 3′-UTR *+820A > G* SNP may theoretically express less IGF-II [22]. Association of HBV clearance with the P1 promoter polymorphism *+6815A > T* may present a functional influence on transcription, too [22].

Endogenous IGF-II functions as a critical survival factor in some cells, during the transition from proliferation to differentiation. The endogenous and exogenous IGF-II were also found to be cytoprotective against cytokine-induced cell apoptosis [31–34]. In one study, the programmed cell death induced by serum deprivation was shown to be reversible by the simultaneous addition of

IGF-II [35]. Therefore, it is conceivable that different serum or intrahepatic IGF-II levels might influence HBV clearance mediated by T-lymphocyte or cytokine-induced hepatocyte apoptosis. In the present study, the *+820A/A* genotype, which has been related with significantly higher IGF-II levels than in *+820G/G* homozygotes [30], showed strong associations with chronic HBV infection. This finding might be explained by anti-apoptotic paracrine or autocrine effects of liver producing IGF-II on HBV infected hepatocytes against the effects of cytokines produced by T-lymphocytes.

On the other hand, overexpression of IGF-II has also been observed in a wide variety of other malignancies [36]. IGF-II has been implicated in oncogenesis, exerts its hormonal, paracrine, and autocrine bioactivities on hepatocarcinogenesis [24], and elevated expression of IGF-II is responsible for accelerated hepatocyte proliferation [37]. A previous study found *IGF2* gene activation and its overexpression in human HCC cell lines, in hepatitis B, HBV-related cirrhosis, and in HCC tissues [38]. In view of the anti-apoptotic and proliferative activities of IGF-II, we are unable to offer an explanation concerning the higher HCC risk associated with *IGF2 + 820G/G*, which is a known low IGF-II producing genotype. Undoubtedly, it is possible that some of the associations determined in the present study were caused by other confounding genetic or environmental factors. The precise role of the *IGF2* genotypes may be resolved by functional studies of IGF2 polymorphisms, which clarify direct causal relationships in the future. While the elucidation of the possible functional roles of these SNPs awaits further study, the existence of independent positive associations between SNPs in the *IGF2* gene and HBV clearance and HCC risk serve as important genetic markers that determine HBV clearance or HCC occurrence.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2006.05.080.

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